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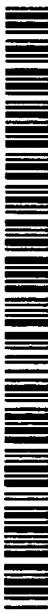
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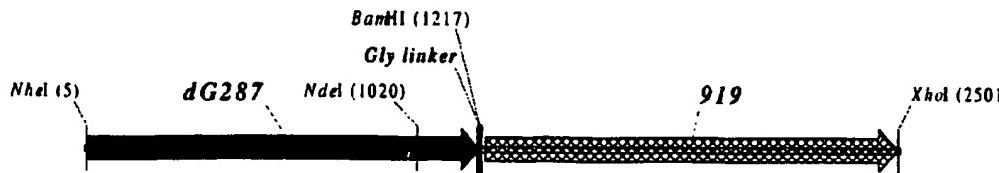
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A2

(54) Title: HYBRID EXPRESSION OF NEISSERIAL PROTEINS

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(57) Abstract: Two or more Neisserial proteins (e.g. A and B) are expressed as a single hybrid protein which can be represented simply by the formula NH₂-A-B-COOH.

HYBRID EXPRESSION OF NEISSERIAL PROTEINS

All documents cited herein are incorporated by reference in their entirety.

TECHNICAL FIELD

This invention is in the field of protein expression. In particular, it relates to the heterologous
5 expression of proteins from *Neisseria* (e.g. *N.gonorrhoeae* or, preferably, *N.meningitidis*).

BACKGROUND ART

International patent applications WO99/24578, WO99/36544, WO99/57280 and
WO00/22430 disclose proteins from *Neisseria meningitidis* and *Neisseria gonorrhoeae*.
These proteins are typically described as being expressed in *E.coli* (i.e. heterologous
10 expression) as either N-terminal GST-fusions or C-terminal His-tag fusions, although other
expression systems, including expression in native *Neisseria*, are also disclosed.

It is an object of the present invention to provide alternative and improved approaches for
the heterologous expression of these proteins. These approaches will typically affect the
level of expression, the ease of purification, the cellular localisation of expression, and/or the
15 immunological properties of the expressed protein.

DISCLOSURE OF THE INVENTION

In accordance with the invention, two or more (e.g. 3, 4, 5, 6 or more) proteins of the
invention are expressed as a single hybrid protein. It is preferred that no non-Neisserial
fusion partner (e.g. GST or poly-His) is used.

20 This offers two advantages. Firstly, a protein that may be unstable or poorly expressed on its
own can be assisted by adding a suitable hybrid partner that overcomes the problem.
Secondly, commercial manufacture is simplified – only one expression and purification need
be employed in order to produce two separately-useful proteins.

Thus the invention provides a method for the simultaneous heterologous expression of two
25 or more proteins of the invention, in which said two or more proteins of the invention are
fused (i.e. they are translated as a single polypeptide chain).

The method will typically involve the steps of: obtaining a first nucleic acid encoding a first
protein of the invention; obtaining a second nucleic acid encoding a second protein of the

invention; ligating the first and second nucleic acids. The resulting nucleic acid may be inserted into an expression vector, or may already be part of an expression vector.

Where just two proteins are joined, the hybrid protein can be represented simply by the formula NH₂-A—B-COOH. A and B can each be selected from any Neisserial proteins, and 5 in particular those represented by SEQ#s 1-4326. The method is well suited to the expression of proteins orf1, orf4, orf25, orf40, Orf46/46.1, orf83, 233, 287, 292L, 564, 687, 741, 907, 919, 953, 961 and 983.

The 42 hybrids indicated by 'X' in the following table of form NH₂-A—B-COOH are preferred:

↓A : B→	ORF46.1	287	741	919	953	961	983
ORF46.1		X	X	X	X	X	X
287	X		X	X	X	X	X
741	X	X		X	X	X	X
919	X	X	X		X	X	X
953	X	X	X	X		X	X
961	X	X	X	X	X		X
983	X	X	X	X	X	X	

- 10 Preferred proteins to be expressed as hybrids are thus ORF46.1, 287, 741, 919, 953, 961 and 983. These may be used in their essentially full-length form, or poly-glycine deletions (ΔG) forms may be used (e.g. ΔG -287, ΔG Tbp2, ΔG 741, ΔG 983 etc.), or truncated forms may be used (e.g. $\Delta 1$ -287, $\Delta 2$ -287 etc.), or domain-deleted versions may be used (e.g. 287B, 287C, 287BC, ORF46₁₋₄₃₃, ORF46₄₃₃₋₆₀₈, ORF46, 961c etc.) and so on.
- 15 Particularly preferred are: (a) a hybrid protein comprising 919 and 287; (b) a hybrid protein comprising 953 and 287; (c) a hybrid protein comprising 287 and ORF46.1; (d) a hybrid protein comprising ORF1 and ORF46.1; (e) a hybrid protein comprising 919 and ORF46.1; (f) a hybrid protein comprising ORF46.1 and 919; (g) a hybrid protein comprising ORF46.1, 287 and 919; (h) a hybrid protein comprising 919 and 519; and (i) a hybrid protein 20 comprising ORF97 and 225.

Further embodiments are shown in the drawings and include ΔG 287-919, ΔG 287-953, ΔG 287-961, ΔG 983-ORF46.1, ΔG 983-741, ΔG 983-961, ΔG 983-961C, ΔG 741-961, ΔG 741-961C, ΔG 741-983, ΔG 741-ORF46.1, ORF46.1-741, ORF46.1-961, ORF46.1-961C,

961-ORF46.1, 961-741, 961-983, 961C-ORF46.1, 961C-741, 961C-983, 961CL-ORF46.1, 961CL-741, and 961CL-983.

Where 287 is used, it is preferably at the C-terminal end of a hybrid; if it is to be used at the N-terminus, it is preferred to use a ΔG form of 287 is used (e.g. as the N-terminus of a hybrid with ORF46.1, 919, 953 or 961).

Where 287 is used, this is preferably from strain 2996 or from strain 394/98.

Where 961 is used, this is preferably at the N-terminus. Domain forms of 961 may be used.

Alignments of polymorphic forms of ORF46, 287, 919 and 953 are disclosed in WO00/66741. Any of these polymorphs can be used according to the present invention.

10 Preferably, the constituent proteins (A and B) in a hybrid protein according to the invention will be from the same strain.

The fused proteins in the hybrid may be joined directly, or may be joined via a linker peptide e.g. via a poly-glycine linker (i.e. G_n where $n = 3, 4, 5, 6, 7, 8, 9, 10$ or more) or via a short peptide sequence which facilitates cloning. It is evidently preferred not to join a ΔG protein 15 to the C-terminus of a poly-glycine linker.

The fused proteins may lack native leader peptides or may include the leader peptide sequence of the N-terminal fusion partner.

Host

It is preferred to utilise a heterologous host. The heterologous host may be prokaryotic or 20 eukaryotic. It is preferably *E.coli*, but other suitable hosts include *Bacillus subtilis*, *Vibrio cholerae*, *Salmonella typhi*, *Salmonella typhimurium*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Neisseria lactamica*, *Neisseria cinerea*, *Mycobacteria* (e.g. *M.tuberculosis*), yeast etc.

Vectors, hosts etc.

25 As well as the methods described above, the invention provides (a) nucleic acid and vectors useful in these methods (b) host cells containing said vectors (c) proteins expressed or expressable by the methods (d) compositions comprising these proteins, which may be suitable as vaccines, for instance, or as diagnostic reagents, or as immunogenic compositions (e) these compositions for use as medicaments (e.g. as vaccines) or as diagnostic reagents (f)

the use of these compositions in the manufacture of (1) a medicament for treating or preventing infection due to Neisserial bacteria (2) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised against Neisserial bacteria, and/or (3) a reagent which can raise antibodies against Neisserial bacteria and (g) a method of treating a patient, comprising administering to the patient a therapeutically effective amount of these compositions.

Sequences

The invention also provides a protein or a nucleic acid having any of the sequences set out in the following examples. It also provides proteins and nucleic acid having sequence identity to these. As described above, the degree of 'sequence identity' is preferably greater than 50% (eg. 60%, 70%, 80%, 90%, 95%, 99% or more).

Nomenclature herein

The 2166 protein sequences disclosed in WO99/24578, WO99/36544 and WO99/57280 are referred to herein by the following SEQ# numbers:

Application	Protein sequences	SEQ# herein
WO99/24578	Even SEQ IDs 2-892	SEQ#s 1-446
WO99/36544	Even SEQ IDs 2-90	SEQ#s 447-491
WO99/57280	Even SEQ IDs 2-3020 Even SEQ IDs 3040-3114 SEQ IDs 3115-3241	SEQ#s 492-2001 SEQ#s 2002-2039 SEQ#s 2040-2166

In addition to this SEQ# numbering, the naming conventions used in WO99/24578, WO99/36544 and WO99/57280 are also used (e.g. 'ORF4', 'ORF40', 'ORF40-1' etc. as used in WO99/24578 and WO99/36544; 'm919', 'g919' and 'a919' etc. as used in WO99/57280).

The 2160 proteins NMB0001 to NMB2160 from Tettelin *et al.* [Science (2000) 287:1809-1815] are referred to herein as SEQ#s 2167-4326 [see also WO00/66791].

The term 'protein of the invention' as used herein refers to a protein comprising:

- (a) one of sequences SEQ#s 1-4326; or
- (b) a sequence having sequence identity to one of SEQ#s 1-4326; or
- (c) a fragment of one of SEQ#s 1-4326.

The degree of ‘sequence identity’ referred to in (b) is preferably greater than 50% (e.g. 60%, 70%, 80%, 90%, 95%, 99% or more). This includes mutants and allelic variants [e.g. see WO00/66741]. Identity is preferably determined by the Smith-Waterman homology search algorithm as implemented in the MPSRCH program (Oxford Molecular), using an affine gap 5 search with parameters *gap open penalty=12* and *gap extension penalty=1*. Typically, 50% identity or more between two proteins is considered to be an indication of functional equivalence.

The ‘fragment’ referred to in (c) should comprise at least *n* consecutive amino acids from one of SEQ#s 1-4326 and, depending on the particular sequence, *n* is 7 or more (e.g. 8, 10, 10 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100 or more). Preferably the fragment comprises an epitope from one of SEQ#s 1-4326. Preferred fragments are those disclosed in WO00/71574 and WO01/04316.

Preferred proteins of the invention are found in *N.meningitidis* serogroup B.

Preferred proteins for use according to the invention are those of serogroup B *N.meningitidis* 15 strain 2996 or strain 394/98 (a New Zealand strain). Unless otherwise stated, proteins mentioned herein are from *N.meningitidis* strain 2996. It will be appreciated, however, that the invention is not in general limited by strain. References to a particular protein (e.g. ‘287’, ‘919’ etc.) may be taken to include that protein from any strain.

It will be appreciated that references to “nucleic acid” includes DNA and RNA, and also 20 their analogues, such as those containing modified backbones, and also peptide nucleic acids (PNA) etc.

BRIEF DESCRIPTION OF DRAWINGS

Figures 1 to 26 show hybrid proteins according to the invention.

MODES FOR CARRYING OUT THE INVENTION

25 Example 1 – hybrids of ORF46

The complete ORF46 protein from *N.meningitidis* (serogroup B, strain 2996) has the following sequence:

30 1 LGISRKISLI LSILAVCLPM HAHASDLAND SFIRQVLDRQ HFEPDGKYHL
 51 FGSRGELAER SGHIGLKGKIQ SHOLGNLMIQ QAAIKGNIGY IVRFSDHGHE
 101 VHSPFDNHAS HSDSDEAGSP VDGFSLYRIH WDGYEHHPAD GYDGPQGGGY

5

151 PAPKGARDIY SYDIKGVAQN IRLNLTDNRS TGQRLADRFH NAGSMLTQGV
 201 GDGFKRATRY SPELDRSGNA AEAFTNGTADI VKNIIGAAGE IVGAGDAVQG
 251 ISEGSNLAVM HGLGLLSTEN KMARINDLAD MAQLKDYAAA AIRDWAVQNP
 301 NAAQGIEAVS NIFMAAIPIK GIGAVRGKYG LGGITAHPIK RSQMGAIALP
 351 KGKSAVSDNF ADAAYAKYPS PYHSRNIRSN LEQRYGKENI TSSTVPPSNG
 401 KNVKLADQRH PKTGVPDFGK GFPNFEKHVK YDTKLIDIQEL SGGGIPKAKP
 451 VSDAKPRWEV DRKLNLITTR EQVEKNVQEI RNNGNKNSNFS QHAQLEREIN
 501 KLKSADEINF ADGMGKFTDS MNDKAFSRLV KSVKENGFTN PVVEYVEING
 551 KAYIVRGNR VFAAEYLGRI HELFKKKVDF PVPNTSWKNP TDVLNESGNV
 10 601 KRPYRISK*

The leader peptide is underlined.

The sequences of ORF46 from other strains can be found in WO00/66741.

15 ORF46 has been fused at its C-terminus and N-terminus with 287, 919, and ORF1. The hybrid proteins were generally insoluble, but gave some good ELISA and bactericidal results (against the homologous 2996 strain):

Protein	ELISA	Bactericidal Ab
Orf1-Orf46.1-His	850	256
919-Orf46.1-His	12900	512
919-287-Orf46-His	n.d.	n.d.
Orf46.1-287His	150	8192
Orf46.1-919His	2800	2048
Orf46.1-287-919His	3200	16384

For comparison, 'triple' hybrids of ORF46.1, 287 (either as a GST fusion, or in Δ G287 form) and 919 were constructed and tested against various strains (including the homologous 2996 strain) versus a simple mixture of the three antigens. FCA was used as adjuvant:

	2996	BZ232	MC58	NGH38	F6124	BZ133
Mixture	8192	256	512	1024	>2048	>2048
ORF46.1-287-919his	16384	256	4096	8192	8192	8192
Δ G287-919-ORF46.1his	8192	64	4096	8192	8192	16384
Δ G287-ORF46.1-919his	4096	128	256	8192	512	1024

20 Again, the hybrids show equivalent or superior immunological activity.

Hybrids of two proteins (strain 2996) were compared to the individual proteins against various heterologous strains:

	1000	MC58	F6124 (MenA)
ORF46.1-His	<4	4096	<4
ORF1-His	8	256	128
ORF1—ORF46.1-His	1024	512	1024

Again, the hybrid shows equivalent or superior immunological activity.

Example 2 – hybrids of ΔG287

The deletion of the (Gly)₆ sequence in 287 was found to have a dramatic effect on protein expression. The protein lacking the N-terminal amino acids up to GGGGGG is called ‘ΔG287’. In strain MC58, its basic sequence (leader peptide underlined) is:

5

```
SPDVKS ADTLSKPAAP VVSEKETEAK EDAPQAGSQG QGAPSAQGSQ DMAAVSEENT
GNNGAVTADN PKNEDEVAQN DMPQNAAGTD SSTPNHTPDP NMLAGNMENQ ATDAGESSQP
ANQPDMANAA DGMQGDDPSA GGQNAGNTAA QGANQAGNNQ AAGSSDPIPA SNPAPANGGS
NFGRVVDLNG VLIDGPSQNI TLTHCKGDSC SGNNFLDEEV QLKSEFEKLS DADKISNYKK
10 DGKNDKFVGL VADSVQMKGI NQYIIFYKPK PTSFARFRS ARSRSRSLPAE MPLIFVNQAD
TLIVDGEAVS LTGHSGNIFA PEGNYRYLTY GAELPLGGSY ALRVQGEPAK GEMLAGAAVY
NGEVLHFHTE NGRPYPTRGR FAAKVDFGSK SVDGIIDSGD DLHMGQTQKFK AAIDGNGPKG
TWTENGSGDV SGKFYGPAGE EVAGKYSYRP TDAEKGGFGV PAGKKEQD*
```

- 15 ΔG287, with or without His-tag (‘ΔG287-His’ and ‘ΔG287K’, respectively), are expressed at very good levels in comparison with the ‘287-His’ or ‘287^{untagged}.

On the basis of gene variability data, variants of ΔG287-His were expressed in *E.coli* from a number of MenB strains, in particular from strains 2996, MC58, 1000, and BZ232. The results were also good – each of these gave high ELISA titres and also serum bactericidal

- 20 titres of >8192. ΔG287K, expressed from pET-24b, gave excellent titres in ELISA and the serum bactericidal assay.

25 Deletion of poly-Gly sequences is also applicable to Tbp2 (NMB0460), 741 (NMB 1870) and 983 (NMB1969). When cloned in pET vector and expressed in *E.coli* without the sequence coding for their leader peptides and without poly-Gly (*i.e.* as “ΔG forms”), the same effect was seen – expression was good in the clones carrying the deletion of the poly-glycine stretch, and poor or absent if the glycines were present in the expressed protein.

ΔG287 was fused directly in-frame upstream of 919, 953, 961 (sequences shown below) and ORF46.1:

30

ΔG287-919
ATGGCTAGCCCCGATGTTAAATCGCGGACACGCTGTCAAAACCGGCCCTCCTGTTGCTGAAAAAGAGACAGAG
GTAAAAGAAGATCGCCACAGGCAGGTTCTCAAGGACAGGGCGCGCATCCACACAAGGAGCCAAGATATGGCGGA
GTTTCGGCAAAAAATACAGGCAATTGGCGGTGCGGCAACACGGACAAACCCAAAAATGAAGACGAGGGACCGCAA
GATATGCCGCAAAATTCGCCGAATCCGCAAATCAAAACAGGGAACACAACCAACCCGCCGATTCTCAGATTCCGCCCC
25 GCGTCAAAACCTGCACCTGCAATGGCGTAGCAATTGGAGGGTTGATTGGCTAATGGCTTTGATTGATGGG
CCGTCGCAAAATATAACGTTGACCCACTGTAAAGGGGATTCTTGTAATGGTGTATAATTGATGAAGAACGACCC
TCAAAATCAGAAATTGAAAATTAAAGTCTGAACGAAATTGAGAAATATAAGAAAGATGGGAAAGCGATAAAATT
35 ACTAAATTGGTTGCGACAGCAGTTCAAGCTAATGGAAACTAAACAAATTGTCATCATTTATAAAAGACAAGTCCGCTTC
TCTTCATCTCGCGGATTCAAGCGTTCTGCACGGTCGAGGGTCGCTTCTGCCGAGATGCCCTAATCCCCGTC
CAGGGGATACGCTGATTGTCGATGGGGAGCGGTCAAGCTGACCGGGCATTCGGCAATATCTTCGGCCCCGAAGGG
40 AATTACCGGTATCTGACTTACGGGGGGAAAAATTGCGCGGATCGTATGCCCTCCGTGTGCAAGGGCAACCGGCA
AAAGGCAGGATGCTTGGCGACGGCGTGTACAACGGCGAAGTGTGCAATTTCATACGGAAAACGGCCGCTCGTAC
CCGACTAGAGGGCAGGTTGGCGCAAAAGTCGATTCGGCAGCAAATCTGTGGACGGCATTATCGACAGCGGCGATGAT
TTGCATATGGGTACGCAAAATTCAAGCCGCATCGATGAAACGGCTTAAAGGGACTTGGACGGAAAATGGCGGC
GGGGATGTTCCCGAAGGTTTACGGCCGGCCGGAGGAAGTGGCGGGAAAATACAGCTATGCCCGACAGATGCG

GAAAAGGGCGGATTCCGGGTGTTGCCGGCAAAAAGAGCAGGATGGATCCGGAGGAGGAGGATGCCAAGCAAGAGC
 ATCCAAACCTTCCGCAACCCGACACATCCGTATCAACGCCCGGACCGCCGGTCGGCATCCCCGACCCGCCGA
 5 ACGACGGTCGGCGCGGGGGCGCTATAACCGTGTACCGCACCTGCCCCACTGGCGGCAGGATGTCAGGCCAA
 GCCAAAAGCCTGCAATCCTCCGCTCGGCTGCCAATTGAAAAACGCCAAGGCTGGCAGGATGTCAGGCCAA
 GCCTTCAACCCCCGTCATCCCTTCAGGCAAAACAGTTTTGAAACGCTATTTCACGCCGTGGCAGGTTGCAAGGC
 AACCGGAAGGCTTGCCTACGGTACCGCTATTACGAGCCGGTCTGAAGGGCAGCAGGCCACAAGCC
 CGCTTCCCATTACGGTATTCCCGAGGATTITATCTCCGCTCCCGCTGCCGTTGCGGAGCGGAAAAGCCCTT
 GTCCGCATCAGGCGAGCGGGAAAAAACCGGGCACAATCGACAATACCGCCGGCACACATACCGCCGACCTCTCCGA
 10 TTCCCCATCACCGCGCGACAACGGCAATCAAAGGAGGTTGAAGGAAGCCGCTTCCCTTACACACGCCAAC
 CAAATCAACGGCGCGCGCTTGACGGCAAGGCCGATACTCGGTTACGCCAAGAGCCGCTGAACTTTTTATG
 CACATCCAAGGCTCGGCCGTCTGAAAACCCCGTCCGGCAATACATCCGATCGGCTATGCCGACA
 CCCTACGTTCCATCGACGCTATATGGCGACAAGGCTACCTCAAGCTCGGCAAGACCTCGATGCAAGGCCATCAA
 GCCTATATGCGGCAAATCGCAACGCCCTCGCGAAGTTGGGTCAAACCCCCAGCTATATCTTTTCCGAGGCTT
 15 GCCGGAAGCAGCAATGACGGTCCCGTCGGCGCACTGGGACGCCGTTGATGGGGATATGCCGCACTGACCGG
 CACTACATTACCTGGCGCGCCCTTATTGTCGCCACCGCCCATCCGGTTACCCGAAAGCCCTCAACGCCGCTG
 ATGGCGCAGGATACCGGCAGCGCATTAAAGGCGGGTGCCTGGATTATTTGGGTACGGCAGCTCCTACCC
 GAACTTGCGGGCAAACAGAAAACCACGGTTACGTCTGGCAGCTCCTACCCAACGGTATGAAGCCGAATACCGCCG
 TAACTCGAG

20	1	MASPDVKSAD TLSKPAAPVV AEKETEVKED APQAGSQGQG APSTQGSQDM
	51	AAVSAENTGN GGAATTDKPK NEDEGPQNDM PQNSAESANQ TGNNQPADSS
	101	DSAPASNAP ANGGSNFGRV DLANGVLIDG PSQNITLTHC KGDSCNGDNL
	151	LDEEAPSKSE FENLNESERI EKYKKDGKSD KFTNLVATAV QANGTNKYVI
25	201	IYKDKSASSS SARFRRSARS RRSILPAEMPL IPVNQADTLI VDGEAVSLTG
	251	HSGNIFAPEG NYRYLTYGAE KLPGGSSYALR VQGEPAKgem LAGTAVYNGE
	301	VLHFHTENGR PYPTGRFAA KVDPFGSKVD GIIDSGDDLH MGTQKFKAII
	351	DGNGFKGTWT ENGGDVSGR FYGPAGEEEVA GKYSYRPTDA EKGGFGVFAF
	401	KKEQDGSSGG GCQKSKIQTFF PQQDTSVING PDRFVGIPDP AGTTVGGGGA
	451	VYTVPFLSL PHWAAQDFAK SLQSFRLGCA NLKNRQGWQD VCAQAFQTPV
30	501	HSFQAKQFFE RYFTPQVAG NGSLAGTVTG YYEPVILKGDD RRTAQARFP I
	551	YGIPDDFISV PLPAGLRSRK ALVRIRQTGK NSGTIDNTGG THTADLSRFP
	601	ITARTTAAIKG RFEGSRFLPY HTRNQINGGA LDGKAPILGY AEDPVBLFFM
	651	HIQGSRLKTH PSGKYIRIGY ADKNEHPPVVS IGRYMADKGY LKLQQTSMQG
35	701	IKAYMRQNPQ RLAEVLQNP SYIFFRELAG SSNDGPVGAL GTPLMGEYAG
	751	AVDRHYITLG APLFVATAHP VTRKALNRLI MAQDTGSAIK GAVRVDYFWG
	801	YGDEAGELAG KQKTTGYVWQ LLPNGMKPEY RP*

ΔG287-953

40 ATGGCTAGCCCCGATGTTAAATCGCGGACACGCTGTCAAAACGGCCCTCTGTTGCTGAAAAAGAGACAGAG
 GTAAAAGAAGATCGCCACAGGCAGGTTCTCAAGGACAGGGCGGCCATCCACACAAGCAGCAAGATATGCCGCA
 GTTTCCGGAGAAAATACAGGCAATGGCGTGCGGCAACAACGGACAAACCCAAAAATGAAGACGAGGGACCGCAA
 45 GATATGCCGCAAATTCGCCGAATCCGCAAATCAAACAGGGAAACAACCAACCCGCCGATTCTCAGATTCCGCCCC
 GCGTCAACCCCTGCACCTCGGAATGGCGTAGCAATTGGGAAGGGTTGATTTGGCTAATGGCTTTGATTGATGGG
 CCCGTCGCAAATATAACCGTGTACCCACTGTAAGGGATTCTGTAATGGTGTATAATTATTGATGAAGAAGCACCG
 TCAAAATCAGAATTGAAAATTAAATGAGTCTGAACGAATTGAGAAAATATAAGGAAAGATGGAAAAGCGATAA
 50 ACTAATTGGTTGCGACAGCAGTTCAAGCTAATGGAACTAACAAATATGTCATCATTATAAAAGACAAGTCCGCTTC
 TCTTCATCTCGCGGATTAGCGTTCTGCACGGTCAGGGAGGTGCTTCTGCGAGGCTGCGCTAATCCCGTC
 CAGCGGATACGCTGATTGCGATGGGAAGCGGTACGGCTGACGGGCAATTCCGCAATATCTCGGCCCGAAGGG
 AATTACCGGTATCTGACTTACGGGGGGAAAATTGCCGGGATCGTATGCCCTCGTGTGCAAGGCAACCGGCA
 55 AAAGCGAAATGTTGGCTGGCACGGCGTGTACAACGGCAAGTGTGCAATTTCATACGGAAAACGGCGTCCGTAC
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 GGGGATGTTCCGGAAGGTTTACGCCCGGCCGGAGGAAAGTGGCGGGAAAATACAGCTATCGCCGACAGATGCG
 60 GAAAAGGGCGGATTCCGGTGTGTTGCCGGCAAAAAGAGCAGGATGGATCCGGAGGAGGAGGCCACCTACAAAGTG
 GACGAATATCACGCCAACGCCGTTCCGCATCGACCATTCAACACCAGCACCAACGTCGGCGTTTACGGTCTG
 ACCGGTTCCGTCGAGTCGACCAAGCAAACCGCACGGTAAAATGACATCACCATCCCGTTGCCAACCTGCAAAGC
 GGTCGCAACACTTACCGACCACTGAAATCAGCGACATCTCGATGCCGCCAATATCCGACATCGCTTGT
 TCCACCAATTCAACTCAACGGCAAAAATCGGTTCCGTTGACGGCAACCTGACCGTACGGCAAAACGCC
 65 GTCAAACCTCAAAGCCGAAAATCAACTGCTACCAAGCCGATGGCAAAACCGAAGTTGCGGGCGACTTCAGC
 ACCACCATCGACCGCACCAATGGGCGTGGACTACCTCGTTACGTTGGTATGACCAAAGCGTCCGACGACATC
 CAAATCGAGGCAGCCAAACAATAACTCGAG

65	1	MASPDVKSAD TLSKPAAPVV AEKETEVKED APQAGSQGQG APSTQGSQDM
	51	AAVSAENTGN GGAATTDKPK NEDEGPQNDM PQNSAESANQ TGNNQPADSS
	101	DSAPASNAP ANGGSNFGRV DLANGVLIDG PSQNITLTHC KGDSCNGDNL
	151	LDEEAPSKSE FENLNESERI EKYKKDGKSD KFTNLVATAV QANGTNKYVI

201 IYKDKSASSS SARFRRSARS RRSLPAEMPL IPVNQADTLI VDGEAVSLTG
 251 HSGNIFAPEG NYRYLTYGAE KLPGGSYALR VQGEPAKgem LAGTAVYNGE
 301 VLHFHTENGR PYPTGRFAA KVDFGSKSVD GIIDSGDDLH MGTQKFKAII
 351 DGNGFKGTWT ENGGGDVSGR FYGPAGEEEVA GKYSYRPTDA EKGPGVFAG
 5 401 KKEQDGSSGG GATYKVDEYH ANARFAIDHF NTSTNVGGFY GLTGSVEFDQ
 451 AKRDGKIDIT IPVANLQSGS QHFTDHLKSA DIFDAAQYPD IRFVSTKFNF
 501 NGKKLVSVDG NLTMHGKTAP VKLKAEKFNC YQSPMAKTEV CGGDFSTTID
 551 RTKWGVVDYLV NVGMTKSVRI DIQIEAAKQ*

10 **ΔG287-961**
 ATGGCTAGCCCCGATGTTAAATCGCCGACACGCTGTCAAACCGGCCGCTCCTGTTGCTGAAAAAGAGACAGAG
 GTAAAAGAAGATGCGCACAGGCAGGTTCTCAAGGACAGGGCGGCCATCCACACAAGGCAAGATATGGCGGA
 15 GTTCGGCAGAAAATACAGGCATGGCGTGGCAACACGGACAAACCCAAAATGAAGACGAGGGACCGCAAAT
 GATATGCCGAAAATTCCGCCAACCGCAATCAAACAGGGACAAACCAACCCGCGATTCTCAGATTCCGCC
 GCGTCAAACCCCTGCACCTGCAATGGCGTAGCAATTGGAAAGGGTTGATTTGGCTAATGGGTTGATGGG
 CCCTCGCAGAAAATATAACGTTGACCAACTGTAAGGCGATTCTGTAATGGTGTAAATTGATGAAGAAC
 TCAAAATCAGAATTGAAAATTAAATGAGTCGAAACGAATTGAGAAATATAAGAAAGATGGAAAAGCGATA
 20 ACTAATTGGTTGCGACAGCAGTCAAGCTAATGGAACTAACAAATATGTCATCATTTATAAAGACAAGT
 TCTTCATCTGCGCGATTCAGCGTTCTGCACGGTCAGGGAGGTCGCTTCTGCCAGGATGCCCTAAT
 CAGGCGGATACGCTGATTGCGATGGGAAAGCGGTGACGCTGACGGGGCATCCGGCAATATCTCG
 AATTACCGGTATCTGACTTACGGGGCGAAAATTGCCC
 25 GCGGATCTGATGGGAAAGGGAAATACAGCTATGCCCTCGTGTGCAAGGCGAAGTGTGCAAGG
 AAAGGCGAAATGCTGCTGGCACGGCGTGTACAACGGCAAGTGTGCAAGGAAACGGCGTCCGTAC
 CCGACTAGAGGCAGGTTGCGCAGGAAAGTCGATTTGGCAGCAATCTGGACGGCATTATCG
 TTGCAATGGGTTACGCAAAATTCAAAGCGCCATCGATGAAACGGCTTTAAGGGACTTGG
 30 GCGGAAATGGGAAAGGGAAATACAGCTATGCCGAGGAGGAGGAGGAGGAGGAGGAGG
 GAAAAGGGCGGATTGCGGTGTTGCGGGCAAAAAGAGCAGGATGGATCCGGAGGAGGAGG
 GATGTTAAAAGCTGCCACTGTGCCATTGCTGCTGCTACAACATGGCAAGAAAT
 35 GAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAGACGCAACTG
 TTTAAAGGCTGGGCTGAAAAAAAGCTGCTGACTAACCTGACGCAACGGCA
 AAAGTAAAAGCTGCGAGAATCTGAAATAGAAAAGCTGATGAAACACCAAGT
 GATGCCGCTCTGGATGCAACCACCAACCGCTTGAAATAATTGGAGAAA
 ACAAAATATGTTGATGAAAATTAGAACGCGTGTGATACCGTC
 40 GAGAACGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG
 ATCGCCGATTGATGAAACCAACACTAAGGCAGACGCAAGCCGCAATGA
 GAAGAACCAAACAAACGTCGATGCCAAGTAAAAGCTGCGAGAAACTG
 ACAGCTAATACTGCAGGCCACAAGGCCAAGCTGCGTGC
 45 AAAGATAATATTGCTAAAAAAGCAACAGTGGCAGCTGTACACCAGAGAAG
 GATGGCTGAAACGCTACTACCGAAAATTGGACACACGCTTCTG
 CGCCTGAACGGTTGGATAAAACAGTGTGAGACCTGCG
 50 51 MASPDVKSAD TLSKPAAPVV AEKETEVKED APQAGSQGQG APSTQGSQDM
 AAVSAENTGN GGAATTDKPK NEDEGPQNDM PQNSAESANQ TGNNQPADSS
 101 DSAPASNPAP ANGGSNFGRV DLANGVLIIDG PSQNITLTHC KGDSNCNDNL
 151 LDEEAPSKSE FENLINESERI EKYKKDGKSD KFTNLVATAV QANGTNKVI
 201 IYKDKSASSS SARFRRSARS RRSLPAEMPL IPVNQADTLI VDGEAVSLTG
 251 HSGNIFAPEG NYRYLTYGAE KLPGGSYALR VQGEPAKgem LAGTAVYNGE
 301 VLHFHTENGR PYPTGRFAA KVDFGSKSVD GIIDSGDDLH MGTQKFKAII
 351 DGNGFKGTWT ENGGGDVSGR FYGPAGEEEVA GKYSYRPTDA EKGPGVFAG
 401 KKEQDGSSGG GATNDDDVKK AATVAAAY NNGQEINGFK AGETIYDIDE
 451 DGTITKKDAT AADVEADDFK GLGLKKVVTN LTKTVNENQ NVDAKVAAE
 501 SEIEKLTTKL ADTDAALADT DAALDAATTNA LNKLGENITT FAEETKTNIV
 551 KIDEKLEAVA DTVDKHAEAF NDIAIDLDET NTKADEAVKT ANEAKQTAEE
 601 TKQNVDAVK AAETAAGKAE AAAGTANTAA DKAEAVAAKV TDIAKADIATN
 651 KDNIAKKANS ADVYTREESD SKFVRIDGLN ATTEKLDTRL ASAEKSIADH
 701 DTRLNGLDKT VSDLRKETRQ GLAEQAALSG LFQPYNVGRF NVTAAVGGYK
 751 SESAVAIGTG FRFTENFAAK AGVAVGTSSG SSAAYHVGVN YEW*

60

-10-

	ELISA	Bactericidal
Δ G287-953-His	3834	65536
Δ G287-961-His	108627	65536

The bactericidal efficacy (homologous strain) of antibodies raised against the hybrid proteins was compared with antibodies raised against simple mixtures of the component antigens (using 287-GST) for 919 and ORF46.1:

	Mixture with 287	Hybrid with ΔG287
919	32000	128000
ORF46.1	128	16000

5 Data for bactericidal activity against heterologous MenB strains and against serotypes A and C were also obtained:

Strain	919		ORF46.1	
	<i>Mixture</i>	<i>Hybrid</i>	<i>Mixture</i>	<i>Hybrid</i>
NGH38	1024	32000	-	16384
MC58	512	8192	-	512
BZ232	512	512	-	-
MenA (F6124)	512	32000	-	8192
MenC (C11)	>2048	>2048	-	-
MenC (BZ133)	>4096	64000	-	8192

The hybrid proteins with Δ G287 at the N-terminus are therefore immunologically superior to simple mixtures, with Δ G287-ORF46.1 being particularly effective, even against heterologous strains. Δ G287-ORF46.1K may be expressed in pET-24b.

10 The same hybrid proteins were made using New Zealand strain 394/98 rather than 2996:

15 **Δ G287NZ-919**
ATGGCTAGCCCCGATGTCAGTCGGCGAACACGCTGTCAAAACCTGCCGCCCTGTTGTTCTGAAAAAGAGACAGAG
GCAAAGGAAGATGCGCCACAGGCAGGTTCTCAAGGCACAGGGCGCCATCCGACAAGGCAGTCAAGATATGGCGCG
GTTTCGGAAGAAAATACAGGCATTGGCGTCCGGCAGCACGGACAAACCCAAAAATGAAGACGAGGGGGCCGAAAAT
GATATGCGC AAAATGCGCCGATACAGATAGTTGACACCGAATCACACCCCGGTTCGAATATGCCGGCCGGAAT
ATGGAAAACCAAGCACC GGATGCCGGGGATCGGAGCAGCCGGAAACCAACCGGATATGGCAAAATACGGGGACGGA
ATGCAGGGTGACGATCCGTCGGCAGGGGGAAAATGCCGGCAATACGGCTGCCAAGGTACAATCAAGCCGAAAAC
AATCAAACGCCGGTTCTCAAAATCTGCCCTTCACCCAAATCCTAGGCCACGAATAGCGTGGTGATTGGAGG
ACGAACGTGGCAATTCTGTGATTTGACGGGGCGTCGCAAAATAACGTTGACCCACTGTAAGCGATTCTGT
AGTGGCAATAATTCTTGTGATGAAGAAGTACAGCTAAAATCAGAATTGAAAAATTAAAGTGTGAGCAGACAAAATAAGT
AATTACAAGAAAAGATGGGAAGAATGACGGGAAGAATGATAAAATTGTCGGTTGGTGCCTGCGATAGTGTGAGCAGATGAAG
GGAATCAATCAATATATTCTTTATAAACCTAAACCCACTTCATTTGCCGCGATTAGCGCTCTGCACGGTCGAGG
CGGTGCGCTCCGGCCGAGATGCCGCTGATTCGGCTCAATCAGGCCGATACGCTGATTTGTCGATGGGAAGCGGTGAGC
CTGACGGGGCATTCCGGCAATATCTTGGCGCCGAAGGGAAATTACCGGTATCTGACTTACGGGGCGAAAATTGCC
GGCGGATCGTATGCCCTCCGTGTCAGGCCAACCTTCAAAAGGCAGATGCTGCCGGCACGGCAGTGTACAACGGC
GAAGTGTGCAATTCTACGGAAAGGCCGCTCCGTCAGAGGGCAGGTTGCGCAGGAAAGTCGATTTCGG
AGCAAATCTGTGGACGGCATTATCGACAGCGGCAGGTTGCGATATGGGTACGCAGGAAATTCAAAGCCGCATCGAT
GGAAACGGCTTAAGGGACTTGGACGGAAAGGGCGGGATGTTCCGGAAAGTTTACGGCCGGCCGGCGAG

5 GAAGTGGCGGGAAAATACAGCTATGCCAACAGATGCGGAAAGGGCGATTGGCGTGTGTTGCCGGAAAAAGAG
 CAGGATGGATCCGGAGGAGGAGATGCCAACAGAAGAGCATCCAAACCTTCCGCAACCGACACATCCGTATCAAC
 GGGCCCGGACCGGCCGGTCGGCATCCCCGACCCCGGAAACGACGGTCGGCGGGGGGGCGTCTATACCGTTGTA
 CGCACCTGCTCCCTGCCCAACTGGCGGCGCAGGATTGCGCAAAGCCTGCAATCCTCCGCTCGGCTGCCAAT
 TTGAAAAACGCCAAGGCTGGCAGGATGTGTGCGCCAAAGCCTTCAAAACCCCGTCCATTCCCTTCAGGAAAACAG
 TTTTTGAACGCTATTTCACGCCGTGGCAGGTTGCAGGCAACGGAAGCCTGCGGTACGGTTACCGGTTACCGGCTATTACGAG
 CGGTGCTGAAGGGCGACGACAGGGCGACGGACAAGCCCGCTTCCCAGTTACGGTATTCCGACGATTTATCTCC
 GTCCCCCTGCTGCCGGTTGCGGAGCGGAAAAGCCCTTGCCGATCAGGCAGACGGGAAAAAACAGCGGACAATC
 10 GACAATACCGCGGACACATACCGCCGACCTCTCCGATTCACCGCGCACAACGGCAATCAAAGGCAGG
 TTTGAAGGAAGCCGCTCCCTCCCTACCACAGCGCAACCAAATCAACGGCGCGCCTGACGGCAAGGCCGATA
 CTGGTTACGCCAAGACCCCGTCAACTTTTTATGCACATCCAAGGCTGGGGCGTCTGAAAACCCCGTCCGGC
 AAATACATCCGATCGCTATGCCGACAAAACGAACATCCCTACGTTCCATCGGACGCTATATGGGGACAAAGGC
 TACCTCAAGCTCGGGCAGACCTCGATGCAAGGCTATATGCCGAAACGCCCTCGGCAACTCCGACTGCCGAAAGTT
 15 TTGGGTCAAACCCAGCTATATCTTTTCCCGAGCTGCGGAAAGCAGCAATGACGGTCCCGTCCGGCAGCTGGC
 ACGCCGTTGATGGGGAAATATGCCGGCGCAGTCGACCGGCACTACATTACCTTGGGGCGGCCCTIATTGTGCCACC
 GCCCATCCGTTACCCGAAAGCCCTCAACCGCCTGATTATGGCGCAGGATACCCGAGCGCAGTAAAGGGCGGGTG
 CGCGTGGATTATTTTGGGATAACGGCAGAAGCCGGCAACTGCCGAAACAGAAAACCACGGGTTACGTCTGG
 CAGCTCCTACCCAACGGTATGAAGCCGAAATACCGCCGTAAGAGCTT
 20 1 MASPDVKSAD TLSKPAAPVV SEKETEAKED APQAGSQGQG APSAQGGQDM
 51 AAVSEENTGN GGAAATDKPK NEDEGAQNDM PQNAADTDSL TPNNTPASN
 101 PAGNMENQAP DAGESEQPAN QPDMANTADG MQGDDPSAGG ENAGNTAAQG
 151 TNQAENNQTA GSQNPPASSTN PSATNSGGDF GRTNVGNSVV IDGPSQNITL
 201 THCKGDSCSG NNFLDEEVQL KSEFEKLSDA DKISNYKKDG KNDGKNDKFV
 25 251 GLVADSVQMK GINQYIIFYK PKPTSFARFR RSARSSRSLP AEMPLIPVNQ
 301 ADTLIVDGEA VSLTGHSNI FAPEGNYRYL TYGAEKLPGG SYALRVQGEPE
 351 SKGEMLAGTA VYNGEVLHFH TENGRRPSPSR GRFAAKVDFG SKSVDGILIDS
 401 GDGLHMGTOPK FKAIDGNGF KGTWTENGGG DVSGKFYGPAA GEVEAGKYSY
 451 RPTDAEKGGF GVFAGKKEQD GSggggcQSK SIQTFPQPDT SVINGPDRPV
 501 GIPDPAGTTV GGGGAVYTVV PHLSPHWAA QDFAKSLQSF RLGCANLKNR
 551 QGWQDVCAQA FQTPVHSFQA KQFFERYFTP WQVAGNGSLA GTVTGYYEPV
 601 LKGDDRRTAQ ARFPIYGIPD DF1SVPLPAG LRSKGALVRI RQTKKNSGTI
 651 DNTGGTHTAD LSRFPITART TAIKGRFEGS RFLPYHTRNQ INGGALDGKA
 701 PILGYAEDPV ELFHMHQGS GRLKTPSGKY IRIGYADKNE HPYVSIGRYM
 751 ADKGYLKLQ TSMQGIKAYM RQNQRLAEV LGQNPQSYIFF RELAGSSNDG
 801 PVGALGTPML GEYAGAVDRH YITLGAPLFV ATAHPTVRKA LNRLIMAQDT
 851 GSAIKGAVRV DYFWGYGDEA GELAGKQKTT GYVWQLLPNG MKPEYRP*

40 AG287NZ-953
 ATGGCTAGCCCGATGTCAGTCGGGGACACGCTGCAAAACCTGCCGCCCTGTTGTTCTGAAAAGAGACAGAG
 GCAAAGGAAGATGCCAACAGGAGGTTCTCAAGGACAGGGCGCGCATCCGACAAGGCGGTCAGGATATGGGGCG
 GTTTCGGAAGAAAATACAGGAATGGCGGTGCGGAGCAACGGACAAACCCAAAATGAAGACGAGGGGGCGCAAAT
 GATATGCCGAAAATGCCGCCATACAGATAGTTGACACCGAATCACACCCCGGCTTCGAATATGCCGCCGGAAT
 45 ATGGAAAACCAAGCACCAGTGGGGGATCGGAGCAGCCGAAACCAACCGGATATGCCAAATACGGGGCG
 ATGCAGGGTGAATCGTCGGCAGGGGGAAAATGCCGCAATACGGCTGCCAAGGTACAAATCAAGCCGAAAAC
 AATCAAACCGCCGGTTCTCAAAATCCTGCCCTTCACCAACCTAGGCCACGAATAGCGGTTGGTGAATTGGAGG
 ACTGAACGTGGCAATTCTGTTGTTGACAGGGCGTCGCAAAATATAACGTTGACCCACTGTAAGGCGATTCTGT
 AGTGGCAATAATTCTTGGATGAAGAAGTACAGCTAAATCAGAAATTGAAATTAAAGTGTGCAAGACAAAATAAGT
 50 AATTACAAGAAAGATGGGAAGAATGACGGGAAGAATGATAAATTGTCGGTTTGGGCGATAGTGTGCA
 GGAATCAATCAATATTATCTTTATAAACCTAAACCACTTCATTGCGCAGTGTGCGTCTGCACGGTCAGG
 CGGTCGCTCCGGCCAGATGCCGCTGATTCCGTCATCAGGCCAGTACGCTGATTGCGTATGGGAAAGGGCG
 CTGACGGGGCATTCCGCAATTCTGCCCAAGGGATTACCGGTATCTGACTTACGGGGGGAAAATGCC
 55 GGCGGATCGTATGCCCGTGTCAAGGGAAACCTTCAGGGAAATGCTCGGGGACGGCAGTGTACAACGGC
 GAAGTGTGTCATTTCTACGGAAAACGGCGTCCGTCGGTCCAGAGGCAAGGTTGCGGCAAAGTCGATTTCGGC
 AGCAAATCTGTGGACGGCAATTACGACAGGGCGATGGTTGCAATAGGGTACGCAAAATCAAGCCGCACTCGAT
 GGAAACGGCTTTAAGGGGACTTGGACGGAAAATGGCGGCGGGATGTTCCGGAAAGTTTACGGCCGGCGAG
 GAAGTGGCGGGAAAATACAGTATGCCAACAGATGCCAAAAGGGCGGATTGGCGTGTGTTGCCGCAAAAAGAG
 CAGGATGGATCCGGAGGAGGAGGCCACCTACAAAGTGGCAAGATACGCCAAACGCCGTTGCCCATCGAC
 60 TTCAACACCAGCACCACGTCGGGTTTACGGTCTGACGGGTTCCGTCAGTTGACCAAGCAAAACGG
 AAAATCGACATCACCATCCCCGTTGCCAACCTGCAAGCGGTTGCAACACTTTACCGACCCACTGAAATCAG
 CGACATCTTCCGATGCCGCCAATATCCGGACATCCGTTGTTCCACCAAATTCAACTCAACGGCAAAACTGG
 GTTGACGGCAACCTGACCATGCAACGGAAAACGCCCGTCAACTCAAAGCCAAAATTCAACTGCTACCAAGC
 CCGATGGCGAAAACCGAAGTTGCGGCGGCGACTTCAGCACCACATGACCGCACCACGCCGCAAAATGG
 65 GGACTACCTGTTGAGCAACAGCGTCGCCATGCACTCCAAATCGAGGCGAGCAACAAATAAGCTT

51 AAVSEENTGN GGAAATDKPK NEDEGAQNDM PQNAADTDSL TPNHTPASNM
 101 PAGNMENQAP DAGESEQPAN QPDmantadG MQGDDPSAGG ENAGNTAAQG
 151 TNQAENNQTA GSQNPASTN PSATNSGGDF GRTNVGNSVV IDGPSQNITL
 201 THCKGDSCSG NNFLEEVQL KSEFEKLSDA DKISNYKKDG KNDGKNDKFV
 251 GLVADSVQMK GINQYIIFYK PKPTSFARFR RSARSRRSLP AEMPLIPVNQ
 301 ADTLIVDGEA VSLTGHSGNI FAPEGNYRYL TYGAEKLPGG SYALRVQGEP
 351 SKGEMLAGTA VYNGEVLHFP TENGRPSPSR GRFAAKVDFG SKSVDGIIDS
 401 GDGLHMGTKQ FKAAIDGNF KGTWTENGG DVSGKFYGP GEEVAGKYSY
 10 451 RPTDAEKGGF GVFAKGKEQD GSggggatYK VDEYHANARF AIDHFNTSTN
 501 VGGFYGLTGS VEFDQAKRDG KIDITIPVAN LQSGSQHFTD HLKSADIFDA
 551 AQYPDIRFVS TKFNPNGKKL VSVDGNLTMH GKTAPVKLKA EKFNCYQSPM
 601 AKTEVCGGDF STTIDRTKWG VDYLNVGMT KSVRIDIQIE AAKQ*

AG287NZ-961

15 ATGGCTAGCCCCGATGTCAGTCAGCTGGCGACACGCTGTCAAAACCTGCCGCCCCCTGTTGTTCTGAAAAAGAGACAGAG
 GCAAAGGAAGATGCGCCACAGGCAGGTTCTCAAGGCAGGGCAGCCATCCGCACAGGCGTCAGATATGGCGCG
 GTTTCGGAAGAAAATACAGGCAATGGCGGTGGCAGCAACGGACAAACCCAAAATGAAGACGAGGGGGCGCAAAT
 GATATGCCGAAAATGCCGCCATACAGATAGTTGACACCGAATCACACCCCGGTTGCAATATGCCGCCGGAAT
 20 ATGGAAAACCAAGCACCGGATGCCGGGGATCGGAGCAGCGGGAAACCAACCGGATATGCCAAATACGGCGGACCGA
 ATGCAGGGTGACGATCCGTGGCAGGGGGAAAATGCCGCAATACGGCTGCCAAGGTACAATCAAGCCGAAAC
 AATCAAACCGCCGGTTCTCAAAATCCTGCCCTTCACCAATCCTAGGCCACGAATAGCGGTTGGTGAATTGGAGG
 ACGAACGTGGGCAATTCTGTTGATTGACGGGCGTCGAAAATATAACGTTGACCCACTGTAAGGCGATTCTGT
 AGTGGCAATAATTCTTGGATGAAGAAGTACAGCTAAATCAGAATTGAAAATTAAGTGTGAGACAAAATAAGT
 25 AATTACAAGAAAGATGGGAAGAATGACGGGAAGAATGATAAAATTGTCGGTTGGTGGCAGTAGTGTGAGATGAAG
 GGAATCAATCAATATTATCTTTATAAACCTAACCCACTTCATTGCGGATTTAGCGTTCTGCACGGTGCAGG
 CGGTCGCTCCGGCCAGATGCCGCTGATTCCCGTCATCAGGCGGATACGCTGATTGTCGATGGGAAGCGGTGAGC
 CTGACGGGGCATTCCGGCAATATCTCGCGCCCGAAGGAATTACCGGTATCTGACTTACGGGGCGAAAATTGCC
 GCGGATCGTATGCCCTCCGTGTCAGGCGAACCTTCAAAAGCGAAATGCTCGGGGACGGCAGTGTACAACGG
 30 GAAGTGCCTGCAATTTCATACGGAAAACGGCCGATTCAGACAGGGCAGGTTTGGCAGAAAGTCGATTTTCG
 AGCAAATCTGTTGGACGGCATATCGACAGGGCAGGTTTGGCATATGGTACGCACAAATTCAAAGCGGCATCGAT
 GGAAACGGCTTAAAGGGACTTGGACGGAAAATGGCGGGGGATGTTCCGAAAGTTACGGCCGGGAGCGGAG
 GAAGTGGGGGAAAATACAGCTATGCCCAACAGATGCCGAAAGGGGGAGTGGTAAAGGCTGCCACTGTGCCATTG
 CAGGATGGATCCGGAGGGAGGAGGCCAACACGACGAGCTTAAAGGCTGGAGAGACCACGACATTGATGAAAG
 35 TACAACAATGGCCAAGAAATCAACGGTTTCAAGCTGGAGAGACCACGACATTGATGAAAGACGGCACAATTAC
 ACCAAAACCGTCAATGAAAACAAACAAACAGCTGATGCCGAAAGTAAAGCTGAGAAATCTGAAATAGAAAAGTAA
 ACCAAGTTAGCAGACACTGATGCCCTTGTGAGATACTGATGCCGCTGTTGATGCAACCCACGCTTGAATAAA
 TTGGGAGAAAATATAACGACATTGCTGAGAGACTAAGACAAATATGCTAAAATTGATGAAAGGAAACCGT
 40 45 GCTGATACCGTCACAAGCATGCCGAAAGCATTCACGATATGCCGATTCTGGATGAAAGCTGAAACACTAAGG
 GAAGCCGTCAAAACGCCAATGAAGCCAAACAGACGGCGAAGAAACCAACACGCTGCAAGCTGAAACACTAAGG
 GCAGAAACTGCAGCAGGCAAAGCCGAGCTGCCGCTGGCACAGCTAATACTGCCAGCCGAAAGCTGCGCT
 GCAAAAGTTACCGACATCAAAGCTGATATCGCTACGAACAAAGATAATTGCTAAAAGCAACAGTGGCAGCG
 TACACCAGAGAAGAGTCTGACAGCAAATTGTCAGAATTGATGGTCTGAAACGCTACTACCGAAAAATTGG
 45 50 GACGCTTCTGCTGAAATCCATTGCCGATCACGATACTGCCCTGAACGGTTGGATAAAACAGTGTGAGACCTG
 AAAAGAACCCGCCAAGGCCCTGCAAGACAAGCCGCTCTCCGGTCTGTTCAACCTTACAACGTTGGCTGG
 GTAAACGGCTGCACTGGCGGCTACAAATCGAATCGGAGCTGCCATCGGTACCGGCTTCCGTTACCGAAA
 GCCGCCAAAGCAGGGTGGCAGTCGGACTTCGTTCTCCGAGCCTACCATGTCGGCTCAATTACGAGTGG
 TAAAAGCTT

50 1 MASPDVKSA DTLSKPAAFPV SEKETEAKED APQAGSQGQG APSAQGGQDM
 51 AAVSEENTGN GGAAATDKPK NEDEGAQNDM PQNAADTDSL TPNHTPASNM
 101 PAGNMENQAP DAGESEQPAN QPDmantadG MQGDDPSAGG ENAGNTAAQG
 151 TNQAENNQTA GSQNPASTN PSATNSGGDF GRTNVGNSVV IDGPSQNITL
 201 THCKGDSCSG NNFLEEVQL KSEFEKLSDA DKISNYKKDG KNDGKNDKFV
 251 GLVADSVQMK GINQYIIFYK PKPTSFARFR RSARSRRSLP AEMPLIPVNQ
 301 ADTLIVDGEA VSLTGHSGNI FAPEGNYRYL TYGAEKLPGG SYALRVQGEP
 351 SKGEMLAGTA VYNGEVLHFP TENGRPSPSR GRFAAKVDFG SKSVDGIIDS
 401 GDGLHMGTKQ FKAAIDGNF KGTWTENGG DVSGKFYGP GEEVAGKYSY
 451 RPTDAEKGGF GVFAKGKEQD GSggggatYK VDEYHANARF AIDHFNTSTN
 501 INGFKAGETI YDIDEDGTIT KKDATAADVE ADDFKGLGLK KVVTNLTKTV
 551 NENKQNVDAK VKAAESEIEK LTTKLADTDA ALADTDAALD ATTNALNKL
 601 ENITTFAEET KTNIVKIDEK LEAVADTVDK HAEAFNDIAD SLDETNKAD
 651 EAIVTANEAK QTAEETKQNV DAKVKAETA AGKAAEAAAGT ANTAADKA
 701 VAAKVTDIKA DIATNKDNIA KKANSADVYT REESDSKFVR IDGLNATTEK
 751 LDTRLASAEC SIADHDTRLN GLDKTVSDLR KETRQGLAEQ AALSGLFQPY
 801 NVGRFNVTA VGGYKSESAB AIGTGFRTF NFAAKAGVAV GTSSGSSAAY
 851 HVGVNYEW*

Example 3 – hybrids of ΔG983

Protein 983 has the following sequence:

983	ΔG983
5 1 MRTTPPTFPPTK TFKPTAMALA VATTLSACLG GGGGGTSAPD FNAGGTGIGS	
51 NSRATTAKSA AVSYAGIKNE MCKDRSMLCA GRDDVAVTDR DAKINAPPPN	
101 LHTGDFPNPN DAYKNLINLK PAIEAGYTGR GVEVGIVDTG ESVGSISFPE	
151 LYGRKEHGYN ENYKNYTAYM RKEAPEPDGGG KDIETASFDDE AVIETEAKPT	
201 DIRHVKEIGH IDLVSHIIGG RSVDRPAGG IAPDATLHIM NTNDETKNEM	
251 MVAAIRNAWV KLGERGVRIV NNSFGTTSR A GTADLFQIAN SEEQYRQALL	
301 DYSGGDKTDE GIRLMQOSDY GNLSYHIRNK NMLFIFSTGN DAQAQPNTYA	
351 LLPFYEKDAQ KGIITVAGVD RSGEKFREM YGEPGTEPLE YGSNHCGITA	
401 MWCLSAPYE SVRFTRTNPI QIAGTSFSAP IVTGTAAALLL QKYPWMSNDN	
451 LRTTLLTTAQ DIGAVGVDSK FGWGLLDAGK AMNGPASFPF GDFTADTKGT	
501 SDIAYSFRND ISGTGGLIKK GGSQQLQLHGN NTYTGKTIIE GGSLVLYGNN	
551 KSDMRVETKG ALIYNGAASG GSLNSDGIVY LADTDQSGAN ETVHIKGSLQ	
601 LDGKGTLVYTR LGKLLKVDGT AIIGGKLYMS ARGKGAGYLN STGRRVPFLS	
651 AAKIGQDYSF FTNIETDGGL LASLDSVEKT AGSEGDTLSY YVRRGNAART	
701 ASAAAHSAPA GLKHAVEQGG SNLENLMVEL DASESSATPE TVETAAADRT	
751 DMPGIRPYGA TFRAAAAVQH ANAADGVRIF NSLAATVYAD STAAHADMQG	
801 RRLKAVSDGL DHNGTGLRVI AQTQQDGGTW EQGGVEGKMR GSTQTVGIAA	
851 KTGENTTAAA TLGMGRSTWS ENSANAKTDS ISLFAGIRHD AGDIGYLKGL	
901 FSYGRYKNSI SRSTGADEHA EGSVNGTLMQ LGALGGVNVP FAATGDLTVE	
951 GGLRYDLLKQ DAFAEKGSAL GWGNSNLTEG TLVGLAGLKL SQPLSDKAVL	
20 1001 FATAFVERDL NGRDVTGFTGATAATGK TGARNMPHTR LVAGLGADVE	
25 1051 FGNGWNGLAR YSYAGSKQYG NHSGRGRGVGY RF*	

ΔG983 thus has the following basic sequence:

30	TSAPD FNAGGTGIGS NSRATTAKSA AVSYAGIKNE MCKDRSMLCA GRDDVAVTDR DAKINAPPPN
35	LHTGDFPNPN DAYKNLINLK PAIEAGYTGR GVEVGIVDTG ESVGSISFPE LYGRKEHGYN ENYKNYTAYM RKEAPEPDGGG KDIETASFDDE AVIETEAKPT DIRHVKEIGH IDLVSHIIGG RSVDRPAGG IAPDATLHIM NTNDETKNEM MVAAIRNAWV KLGERGVRIV NNSFGTTSR A GTADLFQIAN SEEQYRQALL
40	DYSGGDKTDE GIRLMQOSDY GNLSYHIRNK NMLFIFSTGN DAQAQPNTYA LLPFYEKDAQ KGIITVAGVD RSGEKFREM YGEPGTEPLE YGSNHCGITA MWCLSAPYE SVRFTRTNPI QIAGTSFSAP IVTGTAAALLL QKYPWMSNDN LRTTLLTTAQ DIGAVGVDSK FGWGLLDAGK AMNGPASFPF GDFTADTKGT SDIAYSFRND ISGTGGLIKK GGSQQLQLHGN NTYTGKTIIE GGSLVLYGNN
45	KSDMRVETKG ALIYNGAASG GSLNSDGIVY LADTDQSGAN ETVHIKGSLQ LDGKGTLVYTR LGKLLKVDGT AIIGGKLYMS ARGKGAGYLN STGRRVPFLS AAKIGQDYSF FTNIETDGGL LASLDSVEKT AGSEGDTLSY YVRRGNAART ASAAAHSAPA GLKHAVEQGG SNLENLMVEL DASESSATPE TVETAAADRT
50	DMPGIRPYGA TFRAAAAVQH ANAADGVRIF NSLAATVYAD STAAHADMQG RRLKAVSDGL DHNGTGLRVI AQTQQDGGTW EQGGVEGKMR GSTQTVGIAA KTGENTTAAA TLGMGRSTWS ENSANAKTDS ISLFAGIRHD AGDIGYLKGL FSYGRYKNSI SRSTGADEHA EGSVNGTLMQ LGALGGVNVP FAATGDLTVE GGLRYDLLKQ DAFAEKGSAL GWGNSNLTEG TLVGLAGLKL SQPLSDKAVL FATAFVERDL NGRDVTGFTGATAATGK TGARNMPHTR LVAGLGADVE FGNGWNGLAR YSYAGSKQYG NHSGRGRGVGY RF*

ΔG983 was expressed as a hybrid, with ORF46.1, 741, 961 or 961c at its C-terminus:

55	ΔG983-ORF46.1 ATGACTTCTGCGCCGACTTCAATGCAGGCGTACCGGTATCGCAGCAACAGCAGAGCAACACAGCGAACATCAGCA GCAGTATCTTACGCCGTATCAAGAACGAAATGTGCAAAGACAGAACGATGCTCTGTGCCGGTCGGGATGACGTTGCG
60	GTTACAGACAGGGATGCCAAAATCAATGCCCCCCCCCCGAATCTGCATACCGGAGACTTCCAAACCCAATGACGCA TACAAGAACCTCAACCTCAAACTGCAATTGAAGCAGGCTATACAGGACGCGGGTAGAGGTAGGTATCGTCGAC ACAGGCGAACATCCGTCGGCAGCATATCCTTCCGAACTGTATGGCAGAAAAGAACACCGCTATAACGAAAATTACAAA AACTATACGGCGTATATCGGAAAGGAAGCGCTGAAGACGGAGGCGTAAAGACATTGAAGCTCTTCGACGATGAG GCCGTTATAGAGACTGAAGCAAAGCCGACGGATATCCGCCACGTAAAAGAAATCGGACACATCGATTGGTCTCCCAT

ATTATTGGCGGGCGTCCGTGGACGGCAGACCTGCAGGGGTATTCGCCCGATGCGACGCTACACATAATGAATACG
 AATGATGAAACCAAGAACGAAATGATGGTTGCAGCCATCCGCAATGCATGGTCAAGCTGGCGAACGTGGCGTGCGC
 5 ATCGTCATAAACAGTTTGGAACAAACATGAGGCCAGGCCTGCCCACCTTTTCAAATAGCCAATTGGAGGAGCAG
 TACCGCCAAGCGTTGCTGACTATTCCGGCGGTGATAAAAACAGACGAGGGTATCCGCTGATGCAACAGAGCGATTAC
 GGCAACCTGTCCTACCACATCGTAATAAAAACATGCTTTCATCTTTCGACAGCAATGACGCCAAGCTCAGGCC
 AACACATATGCCCTATTGCCATTATGAAAAAGACGCTCAAAAGGCATTATCACAGTCGAGCGTAGACCGCAGT
 GGAGAAAAGTCAACGGGAAATGATGGAGAACCGGGTACAGAACCCCTTGAGTATGGCTCAAACCAATTGGGAATT
 ACTGCCATGTGGTGCCTGCGCACCTATGAAAGCAAGCGTCCGTTACCCGTCACAAACCCGATTCAAATTGCCGA
 10 ACATCTTTCCGACCCATCGTAACCGGCACGGCGCTGCTGAGCAAGTTCGGCTGGACAGCAAGTTCGGCTGGGACTGCTG
 CTGCGTACCCACGTTGCTGACGACGGCTCAGGACATCGGTGAGTCGGCTGGACAGCAAGTTCGGCTGGGACTGCTG
 GATGCGGGTAAGGCCATGAAAGGACCCCGCTTCCGTTCCGACTTACCGGCAAGCTGCTGAGTACGAAAGGTACATCCGAT
 ATTGCTACTCTTCCGTAACGACATTTCAGGCACGGGCCGATCTGCAAGGCGCTGATCAAACAGGCAACTGCAACTGCA
 GGCAACAAACACCTATAACGGCAAAACCAATTATGAAAGGGCGCATCCGGCGCAGCCTGAAACAGGACGGCAATTGCTATG
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 15 CTGGCAGATAACGACCAATCCGGCGAACGAAACGTCACATCAAAGGCACTGCTGAGCGTGGGACGGCAATTGCTAT
 CTGTCAGACACGTTGGCAAACCTGCTGAAAGTGGACGGTACGGGATTATCGGCGCAAGCTGTCAGTGTGCGCACGC
 GGCAAGGGGGCAGGCTATCTCAACAGTACCGGACGACGTTCCCTTCCTGAGTGGCGCAAATCGGGCAGGATTAT
 TCTTTCTTCACAAAACATCGAACCCGACGGCGCTGCTGGCTTCCCTCGACAGCGTCGAAAAAACAGCGGGCAGTGA
 20 GGGCACACGCTGTCCTATTATGTCGTCGCGCAATGCGCACGGACTGCTTCCGAGCGGACATTCCGCCCCGCC
 GGTCGAAACACGCGCTAGAACAGGGCGCACGAACTGAAAGTGGATGGTCGAACTGGATGCTCCGAACTCATCC
 GCAACACCCGAGACGGTTGAAACACTGCGGAGCGACAGATATGCGGGGATCCGCCCCCTACGGCGCAACTTTC
 CGCGCAGCGGAGCGCTACCGGATCGCAATGCCGAGCGGAGCTGACGTTCCCTGAGTGGCGCTACCGTCTAT
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 25 ACGGGCTCGCGCTCATGCCAACACCGAACAGGACGGTGAACGGGAAACAGGGGGTTGAGGCAAATGCGC
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 30 GGAGATTGACGGTCAAGGGCTGCGCTACGACCTGCTAAACAGGATGCAATTGCGGAAACAGGAGTGCATTGCG
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 35 GTGCAATTGGCAACGGCTGGAACGGCTTGGCACGTTACAGCTACGCCGTTCCAAACAGTACGGCAACACAGT
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 45 ACCGAAAAAGAGATGGCGCGCATCACGATTGGCAGATATGGCAGATATGGCGCAACTCAAAGACTATGCC
 GATTGGGAGTCCAAAACCCAAATGCCGACAAGGCTAGGGCACGGATTCAACGGCAGCACCGGACAACGGCTGGCACC
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 TACCATCCCGAAATATCCGCTCAACCTGGAGCAGCGTACGGCAAGAAAACATCACCTCTCAACCGTGCGCG
 50 TCAAAACGGCAAAATGTCAAACTGGCAGACCAACGCCACCCGAGACAGCGTACCGTTGACGGTAAAGGGTT
 AATTGGAGAAGCACGTGAAATATGATACGCTCGAGCACCACCAACCAACTGA

1	MTSAPDFNAG GTGIGSNSRA TTAKSAAVSY AGIKNEMCKD RSLMCAGRDD
51	VAVTDRAKI NAPPNLHTG DFPNPNDAYK NLINLKPJIE AGYTGRGV
101	GIVDTGESEVG SISFPELYGR KEHGYNENYK NYTAYMRKEA PEDGGKDIE
151	ASFDDDEAVIB TEAKPTDIRH VKEIGHIDLV SHIIGRSDV GRPAGGIAPD
201	ATLHIMNTND ETKNEMMVA IRNAWVKLGE RGVRIVNNSF GTTSRAGTAD
251	LFOQIANSEQ YRQALLDYSG GDKTDEGIRL MQQSDYGNLS YHIRKNMLF
301	IFSTGNDAQA QPNTYALLPF YEKDAQKGII TVAGVDRSRE KFKREMYGEP
351	GTEPLEYGSN HCGITAMWCL SAPYEASVRF TRTNPIQIAG TSFSAPIVTG
401	TAALLLQKYP WMSNDNLRTT LLTTAQDICA VGVDSKFGWG LLDAGKAMNG
451	PASFPFDFT ADTKGTSIA YSFRNDISGT GGLIKKGGSQ LQLHGNNTYT
501	GKTIIIEGGSL VLYGNNKSDM RVEVKALIY NGAASGGSLN SDGIVYLADT
551	DQSGANBTvh IKGSLQLDGK GTLYTRLGKL LKVDTGAIIG GKLYMSARGK
601	GAGYLNSTGR RVPPFLSAKI QDYSFFTNI ETDGGLLASL DSVEKTAGSE
651	GDTLSYYVRR GNAARTASAA AHSAPAGLKH AVEQGGSNLE NLMVELDASE
701	SSATPETVET AAADRTDMPG IRPYGATFRA AAAVQHANAA DGVRIFNSLA
751	ATVYADSTAA HADMQRRLK AVSDGLDHNG TGLRVIAQTQ QDGGTWEQGG

5	801 VEGKMRGSTQ TVGIAAKTGE NTTAAATLGM GRSTWSSENSA NAKTDSISLF 851 AGIRHDAGDI GYLKGLF SYG RYKNSISRST GADEHAEGSV NGTLMQLGAL 901 GGVNVPFAAT GDLTVEGGLR YDLLKQDAFA EKGSGALGWSG NSLTTEGTLVG 951 LAGLKLSQLP SDKAVLFATA GVERDLNGRD YTWTGGFTGA TAATGKTGAR
10	1001 NMMPHTRLVAG LGADVEFGNG WNGLARYSYA GSKQYGNHSG RVGVGYRFLD 1051 GGGGTGSSDL ANDSFIRQVL DRQHFEPDGK YHLFGSRGEL AERSGHIGLG 1101 KIQSHQLQNL MIQQAAIKGN IGYIVRFSDH GHEVHSPFDN HASHSDSDEA 1151 GSPVDGFSLY RIHWWDGYEHH PADGYDGPQG GGYPAPKGAR DIYSPDIKGV 1201 AQNIRNLNLTD NRSTGQRLAD RFHNAGSMLT QVGVDGFKRA TRYSPELDRS 1251 GNAAEAFNGT ADIVKNIIGA AGEIVGAGDA VQGISEGSNI AVMHGLLJS 1301 TENKMARIND LADMAQLKDY AAAAIRDWAV QNPNAAQGIE AVSNIFMAAI 1351 PIKGIGAVRG KYGLGGITAH PIKRSQMGAI ALPKGSAVS DNFADAAYAK 1401 YPSPYHSRNI RSNLEQRYGK ENITSSTVPP SNGKNVKLAD QRHPKTGVPF 1451 DGKGFPNFEK HVKYDTLEHH HHHH*

AC883-741

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GCCGCCAAGCAACTCGAGCACCACCAACCACACTGA

	1	MTSAPDFNAG GTGIGSNSRA TTAKSAAVSY AGIKNEMCKD RSMLCAGRDD
5	51	VAVTDRDAKI NAPPPNLHTG DFPNPNDAYK NLINLKPATE AGYTGRGVDEV
	101	GIVDTGESVG SISFPELYGR KEHGYNENYK NYTAYMRKEA PEDGGGKDIE
	151	ASFDEAVIE TEAKPTDIRH VKEIGHIDLV SHIIGGRSDV GRPAGGIAPD
	201	ATLHIMNTND ETKNEMMVAI IRNAWVKLGE RGVRIVNNSF GTTSRAGTAD
	251	LFQIANSEEQ YRQALLDYSG GDKTDEGIRL MQQSDYGNLS YHIRRNKNMLF
10	301	IFSTGNDAQA QPNTYALLPF YEKDAQKGII TVAGVDRSGE KFKREMYGEP
	351	GTEPLEYGSN HCGITAMWCL SAPYRASVRF TRTNPIQIAG TSFSAPIVTG
	401	TAALLLQKYP WMSNDNLRRTT LLTTAQDICA VGVDSKFGWG LLDAGKAMNG
	451	PASFPFGDFT ADTKGTSIA YSFRNDISGT GGLIKKGGSQ LQLHGNNTYT
15	501	GKTIIEGGSL VLYGNNNKSDM RVETKGALIY NGAASGGSLN SDGIVVLADT
	551	DQSGANETVH IKGSSLQDGK GTLYTRLGKL LKVDGTAIIIG GKLYMSARGK
	601	GAGYLNSTGR RVPFLSAKI QDYSFFFTNI ETDDGILLASL DSVEKTAGSE
	651	GDTLSYYVRR GNAARTASAA AHSAPAGLKH AVEQGGSNL NLMVELDASE
	701	SSATPETVET AAADRTDMPG IRPYGATFRA AAAVQHANAA DVGRIFNSLA
20	751	ATVYADSTAA HADMQGRRLK AVSDGLDHNG TGLRVIQTO QDGGTWEQGG
	801	VEGKMRGSTQ TVGIAAKTGE NTTAAATLGM GRSTWSENSA NAKTDISLNF
	851	AGIRHDAGDI GYLKGLFSYG RYKNSISRST GADEHABGSV NGTLMQLGAL
	901	GGVNVPFAAT GDLTVEGGLR YDLLKQDAPA EKGSAALGWSG NSLTEGTLVG
	951	LAGLKLSQLPL SDKAVLFATA GVERDLNGRD YTWTGGFTGA TAATGKTGAR
25	1001	NMPHTRLVAG LGADVEFGNG WNGLARYSYA GSKQYGNHSG RVGVGYRFLE
	1051	GSGGGGVAAD IGAAGLADALT APLDHDKDGL QSLTLDQSVR KNEKLKLAAQ
	1101	GAEKTYGNED SLNTGKLND KVSRFDIFRQ IEVDGQLITL ESGEFQVYKQ
	1151	SHSALTAFQT EQIQDSEHSG KMVAKRQFRI GDIAGEHTSF DKLPEGGRAT
	1201	YRGTAFGSDD AGKKLTYTID FAAKQGNGKI EHLKSPELNV DLAADIKPD
30	1251	GKRHAVISGS VLYNQAEKGS YSLGIFGGKA QEVAGSAEVK TVNGIRHIGL
	1301	AAKQLEHHHH HH*

AG983-961

35	ATGACTTCTGCGCCCGACTTCATGCAAGGGGTACCGGTATCGGCAGCACAGCAGAGCAACACAGCGAAATCAGCA GCAGTATCTTACGCCGGTATCAAGAACGAAATGTGCAAAGACAGAACGATGCTCTGTCGGGTGGGATGACGTTGCG GTTACAGACAGGGATGCCAAATCAATGCCCCCCCCCCGAAATCTGCATACCGGAGACTTTCCAACCCAAATGACGCA TACAAGAAATTGATCAACCTCAACCTGCAATTGAAAGCAGGCTATACAGGACGGGGTAGAGCTAGGTTATCTGTCAC ACAGGCGAATCCGTGGCAGCATATCCTTCCGAACTGTATGGCAGAAAAGAACACGGCTATAACGAAATTACAAA AACTATACGGCGTATATGCGGAAGGAAGCGCCTGAAGACGGAGGGCGTAAAGACATTGAAGCTTCTTCGACGATGAG GCCGTTATAGAGACTGAAGCAAAGCGCAGGATATCCGCCACGTTGCGGCTAAAGAAATCGGACACATCGATTGCTCTCC ATTATTGGCGGGCGTCCGTGGACGGCAGACCTGCAAGGGCTATTGCGCCGATGCGCTACACATAATTGAAATACG AATGATGAAACCAAGAACGAAATGATGGTTCAGCCATCCGAATGCGATGGTCAAGCTGGCGAACGTTGGCGTGC ATCGTCAATAACAGTTTGGAAACAACATCGAGGGCAGGCACTGCCACCTTTCCAATAGCCAATTGCGGAGGAGCAG TACCGCCAAGCGTTGCTGACTATTCCGGCGGTGATAAAACAGACGAGGGTATCCGCCGATGCAACAGAGCGATTAC GGCAACCTGTCTTACACATCGTAATAAAACATGCTTTCATCTTTCGACAGGCAATGACGCCAACAGCTCAGCCC AACACATATGCCATTGCAATTGAAAAAGACGCTCAAAAGGCAATTATCACAGTCCGAGGGTAGACCGCAG GGAGAAAAGTTCAAACGGGAAATGTATGGAGAACCGGGTACAGAACCGCTTGAGTATGGCTCCAACATTGCGGAATT ACTGCCATGTGGTGCCTGTCGGCACCCATGAAAGCAAGCGTCCGTTCACCGTACAAACCCGATTCAAAATTGCC ACATCCTTTCCGACCCATCGTAACGGCACGGCGCTCTGCTGCTGCAAGAAATACCCGTTGAGTGAACAGAGCAAC CTGCGTACCGACGGCTGACGACGGCTCAGGACATCGGTGCACTGGCGTGGACAGCAAGTTCGGCTGGGACTGCTG GATGCGGGTAAGGCCATGAACGGACCCCGCTCCTTCCGTGGCGACTTACCGCCGATACGAAAGGTACATCCGAT ATTGCGTACTCCTTCCGTAACGACATTTCAGGCACGGCGCCCTGATCAAAAAAGGCGCAGCCAATGCAACTGCAC GGCAACACACCTATAACGGGAAAACCATTATCGAAGGGCGTTGCTGTTGAGTGCCTGAGGATATG CGCGTCGAAACCAACGGGCGCTGATTATAACGGGGCGGCATCCGGCGCAGCCTGAAACAGCGACGGCATGTC CTGGCAGATACCGACCAATCCGGCGCAAACGAAACCGTACACATCAAAGGCACTGCGAGCTGGACGGCAAAGGTACG CTGTACACACGTTGGGCAAATGCTGAAAGTGGACGGTACGGCGATTATCGGCGGCAAGCTGTAATGTCGGCACGC GGCAAGGGGGCAGGCTATCTCAACAGTACCGGACGACGTGTTCCCTCTGAGTGCCTGAGGATAT TCTTCTCACAACATCGAAACCGACGGCGCCCTGCTGGCTCCCTGACAGCGTCAAGGGGAGGATTAT GGCGACACGCTGTCCTATTATGTCGCGGGCAATGCGGACGGACTGCTTCGGCAGCGGCACATTCCGCGCCCGCC GGTCTGAAACACGCCGTAGAACAGGGCGGCAGCAATTGGAACACCTGATGCTCCAACTCGGATGCTCCATTCAAC
55	
60	

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 30 1 MTSAPDFNAG GTGIGSNSRA TTAKSAAVSY AGIKNEMCKD RSMLCAGRDD
 51 VAVTDRAKI NAPPNLHTG DFPNPNDAYK NLINLKPAIE AGYTGRGVEV
 101 GIVDTGESVG SISFPELYGR KEHGYNEYK NYTAYMRKEA PEDGGKDIE
 151 ASFDDEAVIE TEAKPTDIRH VKEIGHIDLV SHIIGRSVD GRPAGGIAPD
 201 ATLHIMNTND ETKNEMMVA IRNAWVLGE RGVRIVNNSF GTTSRAGTAD
 251 LFQIANSEEQ YRQALLDYSQ GDKTDEGIRL MQQSVDGNLS YHIRNKNMLF
 301 IFSTGNDQAQ QPNTYALLPF YEKDAQKII TVAGVDRSXE KFKREMYGEP
 351 GTEPLEYGSN HCGITAMWCL SAPYEASVRF TRTNPIQIAG TSFSAPIVTG
 401 TAALLLQKYP WMSNDNLRIT LLITTAQDIGA VGVDSKFGNG LLDAGKAMNG
 451 PASPPFGDFT ADTKGTSdia YSFRNDISGT GGLIKKGGSQ LQLHGNNTYT
 501 GKTIIEGGSL VLYGNNKSDM RVETKGALIY NGAASGGSLN SDGIVYLADT
 551 DQSGANETVH IKGSRLQDGK GTLYTRLGKL LVVDGTAIIIG GKLYMSARGK
 601 GAGYLNSTGR RVPFLSAAKI QDYSFFTNI ETDGGILLASL DSVEKTAGSE
 651 GDTLSYYVRR GNAARTASAA AHSAPAGLKH AVEQGGSNL NLMVELDASE
 701 SSATPETVET AAADRTDMPG IRPYGATFRA AAAVQHANAA DGVRIIFNSLA
 751 ATVYADSTAA HADMQRRLK AVSDGLDHNG TGLRVIAQTQ QDGGTWEQGG
 801 VEGKMRGSTQ TVGIAAKTGE NTTAAATLGM GRSTWSENSA NAKTDSISLF
 851 AGIRHDAGDI GYLKGLFSYG RYKNSISRST GADEHAEGSV NGTLMQLGAL
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 1151 KLTTLKLADTD AALADTDAAL DATTNALNKL GENITTFAAEE TTKTNIVKIDE
 1201 KLEAVADTVD KHAEAFTNDIA DSLDETNKA DEAVKTANEK QTAEETKQN
 1251 VDAKVKAET AAGKAEAAAG TANTAADKAE AVAAKVTDIK ADIATNKDNI
 1301 AKKANSADVY TREESDSKFV RIDGLNATEE KLDTRLASAE KSIADHDTRL
 1351 NGLDKTVSDL RKETRQGLAE QAALSGLFQP YNVGRFNVA AVGGYKSESA
 1401 VAIGTGFRT ENFAAKAGVA VGTSSGSSAA YHVGVNYEWL EHHHHHH*

55 **AG983-961c**
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 15 GGCAGGGGGCAGGCTATCTAACAGTACCGGACGACGTGTTCCCTCGAGTCGGCTGGGAGCAGCTGAGAAAACAGGGGAGGATTAT
 TCTTTCTTACAAAACATCGAACCGACGGCGCTGCTGCTTCCCTGACAGCGTCAAGGGGAGCAGGGCAGCAGGGCAGGGCAGTGA
 GGCAGACCGCTGCTCTTATGTCCTCGCGCAATGCGCACGGACTGTTCCGAGCGGCAATTCCGCGCAGGGCAGGGCAGTGA
 20 GGCACACCCGAGACGGTTGAAACTCCGGCAGGGCACCGAACAGATATGCCGGGAGCGGCTGACGGGAGCAGGGCAGGGCAGGGCAGTGA
 CGCGCAGGGCAGGGTACAGCATGCAATGCCGGGAGCGGCTGACGGGAGCAGGGCAGGGCAGGGCAGGGCAGGGCAGTGA
 GCCGACAGTACCGCCGCCCAGCGGATATGCCGGGAGCGGCTGACGGGAGCAGGGCAGGGCAGGGCAGGGCAGGGCAGTGA
 25 ACGGGTCTGCGCTCATGCCAACACCAACAGGACGGTGAACAGGGGAGCGGCTGAGGCAAGGGGAGCGGCTGAGGCAAGGGCAGGGCAGTGA
 GGCAGTACCCAAACCGCGCATGCCGAAACCGGAAAATACGACAGCAGGGCAGGGCAGGGCAGGGCAGGGCAGGGCAGGGCAGTGA
 AGCACATGGAGCGAAAACAGTGCACATGCAAAACCGCAGGGCAGGGCAGGGCAGGGCAGGGCAGGGCAGGGCAGGGCAGGGCAGTGA
 GATATCGGTATCTAACAGGCTGTTCTCTACAGGAGCTAACAAAACAGCAGGGCAGGGCAGGGCAGGGCAGGGCAGGGCAGGGCAGTGA
 CATGCGGAAGGAGCGCTAACGGCACGGCTGATGCAAGCTGGGGCGACTGGGGGGTGTCAACGTTCCGTTGGCCAGGGCAGGGCAGTGA
 30 GGAGATTTGACGGTGAAGGGGGTCTGCGCTACGACCTGCTCAACAGGAGTGGCATTCGGGAGGGGAGGGCAGGGCAGGGCAGGGCAGTGA
 GGCTGGAGCGCAACAGCCTACTGAAGGCACGGTGGCTGAGCTCGGGGCTGAGAGCTGTCGAACCGGAAACCCCTGAGCGAT
 AAAGCGCTCTGTTGCAACGGGGCGTGGAACCGGACCTGAAACGGGAGCTAACCGGAAACGGGGGGGCTGGGGGCTGGGGGAGCT
 35 GCGCGACTGCAAGCAGGGCAAGACGGGGGAGCGCAATATGCCGACACCCGCTGGTGGGGCTGGGGGCTGGGGGAGCT
 GTCGAATTGGCAACGGCTGGAACGGCTTGGCACGTTACAGCTACGCCGGTCCAAACAGTACGGCAACACAGCGGA
 CGAGTCGGGCTAGGCTACCGGTTCTGAGGGTGGCGAGGCAGTGGATCCGCCACAAACAGCAGGATGTTAAAAAA
 GCTGCCACTGPGGCAATTGCTGCTGCTACAACAAATGGCAAGAAAATCAACGGTTCAAGCTGGAGAGGACATCTAC
 GACATTGATGAAGACGGCAACATTACCAAAAAGACGCAACTGCAGGGATGTTGAAGCCGACGACTTTAAAGGTCTG
 GGTCTGAAAAGTCGACTAACCTGACCAAAACCGTCAATGAAAACAAACAAACAGTCGATGCCAAAGTAAAAGCT
 GCAGAACTGTAATAGAAAAGTTAACACCAAGTTAGCAGACACTGATGCCCTTAGCAGATACTGATGCCCTCTG
 40 GATGCAACCCACCAACGCTTGAATAAATTGGAGAAAATATAACGACATTGCTGAAGAGACTAAGACAAATATCGTA
 AAAATTGATGAAAATTAGAAGCGGCTGGCTGATACCGTCGACAAGCATGCCAAGCATTCAACGATATGCCGATTCA
 TTGGATGAAACCAACACTAAGGCAGACGAAGCGCTAACCGCCAATGAAGCCAACAGACGGCGAAGAAACCAA
 CAAAACGTCGATGCCAAGTAAAAGCTGCAAGAAACTGAGCAGGCAAAGCCGAAGCTGCCGTGCCACAGCTAAACT
 SCAGCCGACAAGGCCGAAGCTGCGCTGCAAAGTTACCGACATCAAAGCTGATATCGCTACGAACAAAGATAATATT
 GCTAAAAAAAGCAACAGTGCCTGGCTACACCAGAGAAGGCTGACAGCAAATTGTCAGAAATTGATGGCTGTAAC
 GCTACTACCGAAAAATTGGACACACCGCTTGGCTCTGCTGAAAATCCATTGCCGATCACGATACTGCCCTGAAACGGT
 TTGGATAAAACAGTGTCAACCTGCCAAAGAAACCGCCAAGGCCCTGAGAACACAAGCCGCGCTCCGGCTGTT
 CAACCTTACACAGTGGGCTCGAGCACCCACCAACCAACTGA

45 1 MTSAPDFNAG GTGIGSNSRA TTAKSAAVSY AGIKNEMCKD RSMLCAGRDD
 51 51 VAVTDRDAKI NAPPNLHTG DFPNPNDAYK NLINLKAIE AGYTRGVVE
 101 101 GIVDTGESVG SISFPELYGR KEHGYNENYK NYTAYMRKEA PEDGGKDIE
 151 151 ASFDEAVIE TEAKPTDIRH VKEIGHIDLV SHIIGGRSVD GRPAGGIAPD
 201 201 ATLHIMNTND ETKNEMMVA IRNAWVKLGE RGVRIVNNSF GTTSRAGTAD
 251 251 LFQIANSEQ YRQALLDYSG GDKTDEGIRL MQQSDYGNLS YHIRKNMLF
 301 301 IFSTGNDQAQ QPNTYALLPF YEKDAQKGII TVAGVDRSGE KFKREMYGEP
 351 351 GTEPLEYGSN HCGITAMWCL SAPYEASVRF TRTNPIQIAQ TSFSAPIVTG
 401 401 TAALLLQKYP WMSNDNLRRT LLTTAQDIGA VGVDSKFGWG LLDAGKAMNG
 451 451 PASFPFGDFD ADTKGTSIDIA YSFRNDISGT GGLIKGGSQ LQLHGNNTYT
 501 501 GKTIIEGSSL VLYGNKSDM RVETKGALIY NGAASGGSL SDGIVYLA
 551 551 DQSGANETVH IKGSLQLDGK GTLYTRLGKL LKVDTGTAIIG GKLYMSARGK
 601 601 GAGYLNSTGR RVPFLSAAKI GDYSSFTNI ETDGGLLASL DSVEKTAGSE
 651 651 GDTLSYYVRR GNAARTASAA AHSAPAGLKHA AVEQGGSNLE NLMVELDASE
 701 701 SSATPETVET AAADRTDMPG IRPYGATFRA AAAVQHANAA DGVRIFNSLA
 751 751 ATVYADSTA HADMQGRRLK AVSDGLDHNG TGLRVIAQTQ QDGGTWEQGG
 801 801 VEGMKMRGSTDQ TVGIAAKTGE NTAAATLGM GRSTWSENSA NAKTDSISLF
 851 851 AGIRHDAGDI GYLKGLFSYG RYKNSISRST GADEHAEGSV NGTLMLQLGAL
 901 901 GGVNVPFAAT GDLTVEGGGLR YDLLKQDABA EKGSALGWSG NSLTGTLVG
 951 951 LAGLKLSQPL SDKAVLIPATA GVERDLNCRD YTWTGGFTGA TAATGKTGAR
 1001 1001 NMPHTRLVAG LGADVEFGNG WNGLARYSYA GSKQYGNHSG RVGVGYRFLE
 1051 1051 GGGGTGSATN DDDVKAATV AIAAAYNNGQ EINGPKAGET IYDIDEDEGTI
 1101 1101 TKKDATAADV EADDFKGLGL KRVTNLTKT VNEKNQNVDA KVKAEESEIE
 1151 1151 KLTTKLADTD AALADTDAAL DATTNALNKL GENITTFAAE TKTNIVKIDE

1201 KLEAVADTVK KHAEEAFNDIA DSDLDETNTKA DEAVKTANEAK QTAEEETKQ
 1251 VDAKVKAET AAGKAEAAAG TANTAADKAE AVAAKVTDIK ADIATNKDNI
 1301 AKKANSADVY TREESDSKFV RIDGLNATTKE KLDTRLASAE KSIADHDTRL
 1351 NGLDKTVSDL RKETRQGLAE QAALSGLFQP YNVGLEHHHH HH*

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Example 4 – hybrids of ΔG741

Protein 741 has the following sequence:

1 VNRTAFCCCLS LTTALILTAC SSGGGGVAAD IGAGLADALT APLDHDKGL
 51 QSLTLDQSVR KNEKLKLAAQ GAEKTYGNND SLNTGKLND KVSRDFDFIRQ
 10 101 IEVDGQLITL ESGEFQVYKQ SHSALTAFQT EQIQDSEHSG KMVAKRQFRI
 151 GDIAGEHTSF DKLPEGGRAT YRGTAFGSDD AGGKLITYTID FAAKQGNNGKI
 201 EHLKSPELNV DLAAADIKPD GKRHAVISGS VLYNQAEKGS YSLGIFGGKA
 251 QEVAGSAEVK TVNGIRHIGL AAKQ*

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ΔG741 thus has the following basic sequence:

VAAD IGAGLADALT APLDHDKGL
 QSLTLDQSVR KNEKLKLAAQ GAEKTYGNND SLNTGKLND KVSRDFDFIRQ
 20 101 IEVDGQLITL ESGEFQVYKQ SHSALTAFQT EQIQDSEHSG KMVAKRQFRI
 GDIAGEHTSF DKLPEGGRAT YRGTAFGSDD AGGKLITYTID FAAKQGNNGKI
 EHLKSPELNV DLAAADIKPD GKRHAVISGS VLYNQAEKGS YSLGIFGGKA
 QEVAGSAEVK TVNGIRHIGL AAKQ*

ΔG741 was fused directly in-frame upstream of proteins 961, 961c, 983 and ORF46.1:

25 **ΔG741-961**
 ATGGTCGCCGCCGACATCGTGGGGCTTGGCAGATGCACATAACCGCACCGCTGACCATAAAAGACAAAGGTTGCAG
 TCTTTGACGCTGGATCAGTCCTCAGGAAAAACGAGAAAACTGAAGCTGGCGACAAGGTGCGAAAAAAACTTATGGA
 AACGGTGACAGCCTCAATACGGGAAATTGAAGAACGACAAGGTGAGCCGTTTCGACTTTATCCGCCAATCGAAGTG
 GACGGGCAGCTCATTAACCTTGGAGAGTGGAGAGTTCCAAGTATAACAAACAAAGCAATTCCGCCCTAACGCCCTTCAG
 ACCGAGCAAATACAAGATTGGAGCATTCCGGAAAGATGGTTGCGAACGCCAGTTCAAGAATCGGCGACATAGCGGGC
 30 GAACATACATCTTTTGACAAGCTTCCCGAAGGGCAGGGCAGGGCAGATATCGCGGGACGGCGTTCCGTTAGACGATGCC
 GGCGGAAAACGACCTACACCATAGATTCCGCCCAAGCAGGGAAACGGCAAAATCGAACATTGAAATGCCAGAA
 CTCAAATGCGACCTGGCCGCCGCGATATCAAGCCGATGGAAAACGCCATGCCGTATCAGCGGTTCCGTCCTTAC
 ACCAAGCCGAGAAAGGCAGTTACTCCCTCGTATCTTGGCGAAAAGCCCAGGAAGTTGCCGCAGCGCGGAAGTG
 35 AAAACCGTAAACGGCATAGCCATATCGGCCATCGGCCACTGCGCCTTGCTGCTACAACAATGGCAAGAAATCAACGGTTTC
 AACGACGACGATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTACAACAATGGCAAGAAATCAACGGTTTC
 AAAGCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAAGACGCAACTGCAGCGATGTTGAA
 GCCGACGACTTTAAAGGTCTGGGTCTGAAAAAAGCTGTGACTAACCTGACCAAAACCGTCAATGAAAACAAACAAAAC
 40 GTCGATGCCAAAGTAAAAGCTGCGAGAATCTGAAATAGAAAAGTTAACACCAAGTGTAGCAGACACTGATGCCGTTTA
 GCAGATACTGATGCCGCTCTGGATGCAACCACCGCTTGATGAAATAATTGGGAGAAAATATAACGACATTGCTGAA
 GAGACTAAGACAAAATATGTTGATGAAAATTAGAAGCCGCTGGCTGATACCCTGCGACAAGCATGCCAAGGCA
 TTCAACGATATGCCGATTCTGGATGAAACCAACACTAAGGCAGACGGCGTCAAAACGCCAATGAAGCCAAA
 45 CAGACGGCCGAAGAACCAACAAACACGTCGATGCCAAAGTAAAAGCTGCGAAACTGCGAGGCAAGCCGAAGCT
 GCCGCTGGCACAGCTAATACTGCAAGCCGACAAGGCCGAAGCTGCTGCAAAAGTTACCGACATCAAAGCTGATATC
 GCTACGAACAAAGATAATATTGCTAAAAAAGCAACAGTGCAGCTGGTACACCAAGAGAAGAGTCTGACAGCAAATT
 GTCAGAAATTGATGGTCTGAACGCTACTACCGAAAAATTGAGACACACGCTTGGCTTCTGCTGAAAATCCATGCCGAT
 50 CACGATACTGCCCTGAAACGGTTGGATAAAACAGTGTGACACCTGCGCAAGAAAACCGCCAAGGCCCTTGCGACAACAA
 GCCGCGCTCTCCGGCTGTTCCAACCTTACAACGTTGGCTGGCTCAATGTAACGGCTGAGTCGGCGCTACAAATCC
 GAATCGGAGTCGCCATCGTACCGGCTTCCGCTTACCGAAAACCTTGGCCCAAAGCAGGCGTGGCAGTCGGCACT
 TCGTCCGGTTCTTCCGAGCCTACCATGTCGGCTCAATTACGAGTGGCTCGAGCACCAACACCACCAACTGA

1 MVAADIGAGL ADALTA PLDH KDKGLQSLTL DQSVRKNEKL KLAQGAEKT
 51 YGNQDSLNTG KLKNDKVSF DFIRQIEVDG QLITLESGEF QVYKQSHSAL
 101 TAFQTEQI QD SEHSGKMVK RQFRIGDIAG EHTSF DKLPE GGRATYRGTA
 151 FGSSDAGGKL TYTIDFAAKQ GNGKIEHLKS PELNVDLAAA DIKP DKGKRA
 201 VISGSVLYNQ AEKGSYSLGI FGGKAQEVAG SAEVKT VNGI RHIGLAAKQL
 251 EGGGGTGSAT NDDDVKAAT VAIAAAYMNG QEINGFKAGE TIYDIDEDEGT
 301 ITKKDATAAD VEADDFKGL LKKVUTNLTK TVNENQNVDAKVKAAESEI
 351 EKLTTKLADT DAALADTDAA LDATMTNALNK LGENJITFAE ETKTNIVKID
 401 EKLEAVADTV DKHAEAFNDI ADSLDETNTK ADEAVKTANE AKQTAEEETKQ

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451 NVDAKVAAE TAAGKAEAAA GTANTAADKA EAVAAKVTDI KADIATNNDN
 501 IAKKANSADV YTREESDSKF VRIDGLNATT EKLDTRLASA EKSIADHDTR
 551 LNGLDKTVSD LRKETRQGLA EQAALSLGFQ PYNVGRFNV AAvggykses
 601 AVAIGTGFRF TENFAAKAGV AVGSSGSSA AYHVGVNYEW LEHHHHHH*

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AG741-961c

10 ATGGTCGCCGCCGACATCGGTGCGGGGCTTCCGATGCACTAACCGCACCGCTCGACCATAAAAGACAAAGGTTGCAG
 AACGGTACAGCCTCAATACGGCAAATTGAAGAACGACAAGGTCAAGCTGGCGGACAAGGTGCGGGAAAAACTTATGGA
 GACGGGCAGCTCATTAACCTGGAGAGTGGAGAGTTCCAAGTATACAAACAAAGCCATTCCGCTTAACCGCCCTTCAG
 ACCGAGCAAATACAAGATTGGAGAGTGGAGAGTTCCAAGTATACAAACAAAGCCATTCCGCTTAACCGCCCTTCAG
 15 GAACATACATCTTGGACAAGCTTCCGAGGGCGACATATCGGGGACGGCGTTCCGCTTACAGACGATGCC
 GGCGGAAAATGACCTACACCATAGATTCCGCGGAAAGCAGGGAAACGGCAAATCGAACATTTGAAATGCCAGAA
 CTCAATGTCGACCTGGGCCGCCGATATCAACGGGATGCAAACCGCATGCCGTCAGCGGTTCCGCTTAC
 AACCAAGCCGAGAAAGGCAGTTACTCCCTCGGTATCTTGGCGGAGAGTGGAGAGTGGAGAGTGGAGAGTGGAGAGT
 AAAACCGTAAACGGCATACGCCATATCGGCCATTGGCGGAAAGCAACTCGAGGGTGGCGGAGGCACTGGATCCGCCACA
 AACGACGACGATGTTAAAAAGCTGCCACTGTGGCATTGCTGCTGCCATAACAAATCAACGGTTTC
 20 AAAGCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAAATTACCAAAAGACGCCACTGAGCCGATGTTGAA
 GCCGACGACTTAAAGGTCTGGGCTGAAAAAGTGTGACTAACCTGACCAAAACCGTCAATGAAAACAAACAAAC
 GTCGATGCCAAGTAAAGCTGCAAGAACATCTGAAATAGAAAAGTAAACAAACCAAGTGTACAGACACTGATGCCCTTA
 GCAGATACTGATGCCGCTCTGGATGCAACCACCAACGCCCTGAAATAATTGGAGAAAATATAACGACATTGCTGAA
 GAGACTAAGACAAATATGTAAGGAAATTAGAAGCCGTTGCTGATACCGTCGACAAGCATGCCAAGCA
 25 TTCAACGATATGCCGATTCACTGGATGAAACCAACACTAAGGACAGACGAAGCCGTCAAAACGCCAATGAGCCAAA
 CAGACGGCCGAGAAACCAAACAAACGGTCACTGGGCTGCAAGTGGAGAGTGGAGAGTGGAGAGTGGAGAGTGGAGAGT
 GCCGCTGGCACAGCTAATACTGAGCCGACAAGGCCAGCTGCTGCAAGTGGAGAGTGGAGAGTGGAGAGTGGAGAGT
 GCTACGAACAAAGATAATATTGCTAAAAAGCAACAGTGGGCTGAGCTGCAACGCCAAGGAAACCCGCAAGGGCTGCA
 30 CACGATACTGCCGCTGAAACGGTTGGATAAAACAGTGTCAACGCCAAGGAAACCCGCAAGGGCTGCA
 GCCGCGCTCCGGTCTGTTCAACCTTACAACGTTGCTGAGCACCACCAACCACTGA

1 MVAADIGAGL ADALTAFLDH KDKGLQSLTL DQSVRKNEKL KLAQQAETK
 51 YGNQDSLNTG KLNNDKVSRF DFIRQIEVDG QLITLESGEF QVYKQSHSAL
 101 TAFQTEQIQLD SEHSKGKMKV RQFRIGDIAG EHTSFDFKLPE GGRATYRGTA
 151 FGSDDAGGKL TYTIDFAAKQ GNKGIEHLKS PELNVDLAAA DIKPDKRHA
 201 VISGSVLYNQ AEKGSYSLGI FGKQAEVAG SAEVKTVNGI RHIGLAKQL
 251 EGGGGTGSAT NDDDVKKAAAT VAIAAAYNNQ QBIINGFKAGE TIYDIDEDEGT
 301 ITKKDATAAD VEADDFKGLG LKKVVTNLTK TVNENQNVD AKVKAEESEI
 351 EKLTTKLADET DAALADTDAA LDATTNALNK LGENITTFAE ETKTNIVKID
 401 EKLEAVADTV DKHAEAFNDI ADSLDETNK ADEAVKTANE AKQTAEEETKQ
 451 NVDAKVAAE TAAGKAEAAA GTANTAADKA EAVAAKVTDI KADIATNNDN
 501 IAKKANSADV YTREESDSKF VRIDGLNATT EKLDTRLASA EKSIADHDTR
 551 LNGLDKTVSD LRKETRQGLA EQAALSLGFQ PYNVGLEHHH HHH*

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AG741-983

50 ATGGTCGCCGCCGACATCGGTGCGGGGCTTCCGATGCACTAACCGCACCGCTCGACCATAAAAGACAAAGGTTGCAG
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 ACCGAGCAAATACAAGATTGGAGAGTGGAGAGTGGAGAGTGGAGAGTGGAGAGTGGAGAGTGGAGAGTGGAGAGTGGAGAGT
 55 GAACATACATCTTGGACAAGCTTCCGAGGGCGACATATCGGGGACGGCGTTCCGCTTACAGACGATGCC
 GGCGGAAAATGACCTACACCATAGATTCCGCGGAAAGCAGGGAAACGGCAAATCGAACATTGAAATGCCAGAA
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 60 ATCAACCTAAACCTGCAATTGAAGCAGGCTATACAGGACGGGGTAGAGGTAGGTATCGTCAACAGGGTATAACAAA
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 CGTTCCGTTGAGCCGACGACCTGCAAGGGCGTATGCGCCGATGCGACGCTACACATAATGAATACGAATGATGAAACC
 65 AAGAACGAAATGATGGTGCAGCCATCCGCAATGCATGGGTCAGCTGGCGACCTTCCAAATAGCCAATTCCGAGGAGCAGTACCGCCAAGCG
 TTGCTGACTATTCCGCGGTGATAAAACAGACGAGGGTATCCGCTGATGCAACAGAGCAGTACCGCCAACCTGTCC

TACCACATCCGTAATAAAAACATGCTTTCATCTTTGACAGGAATGACGCACAAGCTCAGCCAAACACATATGCC
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 5 AACGGGAATGTATGGAGAACCGGTAACAGAACCGCTTGAGTATGGCTCAACCCGATTCAAAATTGCGGAATTACTGCCATGTGG
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 10 TTCCGTAACGACATTTCAGGCACGGGGCTTCCGGCTGATCAAAAAAAGGGCAGCCAACGCAACTGCAACGGCAACAAACACC
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 30 GTCGAAGGGCTGCTGCGCTACGACCTGCTCAACAGGATGCTGGGCGACTGGGCGGTGTCAGCTCCGTTGCGCAACGGGAGATTGACG
 AACAGCCTACTGAGGACGCTGGACTCGGACTCGCAACGGGAGCGACTACACGGTAACGGGGCTTACCGGCGACTGCA
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 AACGGCTGGAACGGCTTGGCACGTTACAGTACGCCGTTCCAAACAGTACGGCAACCACAGGGACGAGTCGGCGTA
 GGCTACCGGTTCTCGAGCACCAACCACCAACTGA

1 MVAADIGAGL ADALTAPLDH KDKGLQSLTL DQSVRKNEKL KLAQQAETK
 51 YGNQDSINTG KLNDKVSRF DFIRQIEVDG QLITLESGEF QVYKQSHSAL
 101 TAFQTEQIJD SEHSKVMVAK RQFRIGDLAG EHTSFIDLPE GGRATYRGTA
 151 PGSDDAGGKL TYTIDFAAKQ GNGKIEHLKS PELNVDLAAA DIKPDKRHA
 201 VISGSVLYNQ AEKGYSLSGI FGGKAQEVAQ SAEVKTVNGI RHIGLAAKQL
 251 EGSGGGTSA PDFNAGGTGI GSNSRATTAK SAAVSYAGIK NEMCKDRSML
 301 CAGRDDVAVT DRDAKINAPP PNLTHTGDFPN PNDAYKNLIN LKPAIEAGYT
 351 GRGVVEGIVD TGESVGSISF PELYGRKEHG YNENYKNTA YMRKEAPEDG
 401 GGKDIEASFD DEAVIETEAK PTDIRHVKEI GHIDLVSHII GGRSVDGRPA
 451 GGIAPDATLH IMNTNDETKN EMMVAIRNA WVKLGERGVR IVNNNSFGITTS
 501 RAGTADLFQI ANSEEQYRQA LLDYSSGGDKT DEGIRLMQQS DYGNLSSYHR
 551 NKNMLPFIYST GNDAAQAPNT YALLPFYEQD AQKGIITVAG VDRSGEKFKR
 601 EMYGEPGTEP LEYGSNHCGI TAMWCLSAPY EASVRFTRTN PIQIAGTSFS
 651 APIVTGTAAL LLQKYPWMSN DNLRPLLTT AQDIGAVGVD SKFGWGLLDA
 701 GKAMNPASF PFGDFTADTK GTSDIAYSFR NDISGTGGLI KKGGSQLQLH
 751 GNNTYTGKTI IEGGSLVLYG NNKSDMRVET KGALIYNGAA SGGSLSNSDGI
 801 VYLAQTQDQSG ANETVHKGSLQLDGKGTL TRLGKLLKVD GTAIIGGKLY
 851 MSARGKGAGY LNSTGRRVPF LSAAKIGQDY SFFTNIETDG GLLASLDSVE
 901 KTAGSEGDYL SYVVRGNAA RTASAAAHS AAGLKHAVEQ GGSNLENLMV
 951 ELDASESSSAT PETVETAAAD RTDMPGIRPY GATFRAAAAV QHANAADGVR
 1001 IFNSLAATVY ADSTAHHADM QGRRLLKAVSD GLDHNGTGLR VIAQTOQDGG
 1051 TWEQGGVEGK MRGSTQTVGI AAKTGETTA AATLGMGRST WSENSANAKT
 1101 DSISLFAGIR HDAGDIGYLN GLFSYGRYKN SISRSTGADE HAEGSVNGL
 1151 MQLGALGGVN VPFAATGDLT VEGGLRYDLL KQDAAEKGS ALGWSGNSLT
 1201 EGTLVGLAGL KLSQPLSDKA VLFATAGVER DLNGRDYTVT GGFTGATAAT
 1251 GKTGARNMPH TRLVAGLGA VEFNGNWNL ARYSYAGSKQ YGNHSGRVGV
 1301 GYRFLEHHHH HH*

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AG741-ORF46.1

ATGGTCGGCCGGACATCGTGGGGCTTGGCGATGCACTAACCGCACCGCTGACCCATAAGACAAAGGTTGCAG
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 AACGGTGACAGCCTCAATACGGGAAATTGAAGAACGACAAGGTGAGCGTTCGACTTTATCCGCAAATCGAAGTG
 65 GACGGGCAGCTCATTACCTTGGAGAGTGGAGAGTCCAAAGTATACAACAAAGCCATTCCGCTTAACCGCCTTCAG
 ACCGAGCAATACAAGATTGGAGCAATTCCGGAAGATGGTGCAGACCGCAGTCAGAATCGCGACATAGCGGGC
 GAACATACATCTTGACAAGCTCCGAAGGCGCAGGCGACATATCGCGGGACGGCGTTGGGTCAGACGATGCC

GGC GGAA ACTGACCTACACCATA GATTCGCCGCAAGCAGGGAAACGGAAAATCGAACATTGAAATGCCAGAA
 CTC AATGTCGACCTGGCCGCCGATATCAAGCCGGATGAAAACGCCATGCCGTATCAGCGGTTCCGTCTTAC
 5 ACCAAGCCGAGAAAGCCAGTTACTCCCTCGGTATCTTGCGGAAAGCCCAGGAAGTGC CGCAGCGGAAGTG
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 TTGGCAAACGATTCCTTATCCGGTACTCGGCTACGCACTGGATGGGAAATACAAAGCCATCAGTTGGGCAACTGATG
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 10 TTCGACAACCATGCCATACCGGATTCGATGAAGCCGGTAGTCCCGTTGACGGATTAGCCTTACCGCATCCAT
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 AGGGATATATACAGCTAGACATAAAAGCGTTGCCAAATATGCCCTCAACCTGACCGACAACCGCAGCACCGG
 15 CAACGGCTTCCGACCGTTCCACAATGCCGTAGTATGCGACGAAGGAGTAGGGGACGGATTCAAACGCCACC
 CGATACAGCCCCGAGCTGGACAGATCGGGCAATGCCGAAAGCCTTCAACGGCACTGCAGAATATCGTTAAAACATC
 ATCGCGCGGCAGGAGAAATTGTCGGCGCAGGGATGCGCTGCAGGGATAAGCGAAGGCTCAAACATTGCTGTATG
 CACGGCTTGGGCTGCTGCTTCCACCGAAACAGATGGCGCCATCACCGATTTGGCAGATATGGCGCACTCAAAGAC
 20 TATGCCGCGACAGCCATCCCGATGGCAGTGGCAGTCCAAAACCCAATGCCACAAGGCATAGAAGCGTCAGCAATATC
 TTTATGGCAGCCATCCCCATCAAAGGATTGGAGCTTCCGGGAAATACGGCTTGGCGGCATCACGGCACATCCT
 ATCAAGCGGTCGAGATGGCGCGATCGCATTCCGAAAGGAAATCCCGCTCAGGACAATTGCGATGCGGCA
 TACGCCAAATACCCGTCCTTACCATCCCGAAATATCCGTTCAAATTGGAGCAGCGTTACGGCAAAGAAAACATC
 ACCTCCTCAACCGTGGCGCGTCAAACGGCAAAATGTCAAACGGCAGACCAACGCCACCCGAAGACAGGCGTACCG
 25 TTTGACGGTAAAGGTTCCGAATTGAGAACGTGAAATATGATACGCTCGAGCACCACCAACCACGTGA
 1 MVAADIGAGL ADALTA PLDH KDKGLQSLTL DQSVRKNEKL KLAAQGA EKT
 51 YGNQDSLNTG KLKN DKV SRS DFIRQIEVDG QLITLESGEF QVYKQSHS AL
 101 TAFQTEQIQD SEHS GK MVAK RQFRIGDIAG EHTSF DKLPE GGRATYR GTA
 151 FGSDDAGGKL TYTIDFAAKQ GNGKIEHLKS PELN VDLAAA DIKP DGKR HA
 201 VISGSV LYNQ AEKG SYSLGI FGGKAQEVAG SAEVKTVNGI RHIGLA AKQL
 251 DGGGGT GSSD LANDS FIR QV LDRQH FEPDG KYHL FGSR GE LAER SGH IGL
 301 GKI QSH QLGN LMIQ QAAIKG NIGYIVRFSD HGHEV HSPFD NHASH SD SDE
 351 AGSPV DGFSL YRIHW DGYEH HPADG YDGPQ GGGY PAPKGA RD IY SYD IKG
 30 401 VAQN IRNL NT DNRST GQR LA DRFH NAGS ML TQGVGDGF KR ATRY SP ELD R
 451 SGNAAE AFNG TADIVK NIIG AAGE IVGAGD AVQG ISEG SN IAVM HGL LLL
 501 STENK MARIN DLADMA QLK D YAAAIRDWA VQNP NAQGI EAVSN IFM AA
 551 IPIKGIGAVR GK YGLGGITA HPIKRSQMG A IALPKG KSAV SDNFADAAYA
 601 KYPSPYHSRN IRSN LEQRYG KENITS STVP PSNG KNV KLA DQRHPKTGVP
 35 651 FDGKGFPNFE KHVKYDTLEH HHHHH*

Example 5 – hybrids of 287

Expression of 287 as full-length with a C-terminal His-tag, or without its leader peptide but with a C-terminal His-tag, gives fairly low expression levels. Better expression is achieved 40 using a N-terminal GST-fusion. As an alternative to using GST as an N-terminal fusion partner, 287 was placed at the C-terminus of protein 919 ('919-287'), of protein 953 ('953-287'), and of proteins ORF46.1 ('ORF46.1-287'). In both cases, the leader peptides were deleted, and the hybrids were direct in-frame fusions.

To generate the 953-287 hybrid, the leader peptides of the two proteins were omitted by 45 designing the forward primer downstream from the leader of each sequence; the stop codon sequence was omitted in the 953 reverse primer but included in the 287 reverse primer. For the 953 gene, the 5' and the 3' primers used for amplification included a *Nde*I and a *Bam*HI restriction sites respectively, whereas for the amplification of the 287 gene the 5' and the 3' primers included a *Bam*HI and a *Xho*I restriction sites respectively. In this way a sequential 50 directional cloning of the two genes in pET21b+, using *Nde*I-*Bam*HI (to clone the first gene) and subsequently *Bam*HI-*Xho*I (to clone the second gene) could be achieved.

The 919-287 hybrid was obtained by cloning the sequence coding for the mature portion of 287 into the *Xho*I site at the 3'-end of the 919-His clone in pET21b+. The primers used for amplification of the 287 gene were designed for introducing a *Sal*I restriction site at the 5'- and a *Xho*I site at the 3'- of the PCR fragment. Since the cohesive ends produced by the *Sal*I and *Xho*I restriction enzymes are compatible, the 287 PCR product digested with *Sal*I-*Xho*I could be inserted in the pET21b-919 clone cleaved with *Xho*I.

The ORF46.1-287 hybrid was obtained similarly.

The bactericidal efficacy (homologous strain) of antibodies raised against the hybrid proteins was compared with antibodies raised against simple mixtures of the component antigens:

	Mixture with 287	Hybrid with 287
919	32000	16000
953	8192	8192
ORF46.1	128	8192

- 10 Data for bactericidal activity against heterologous MenB strains and against serotypes A and C were also obtained for 919-287 and 953-287:

<i>Strain</i>	919		953		ORF46.1	
	<i>Mixture</i>	<i>Hybrid</i>	<i>Mixture</i>	<i>Hybrid</i>	<i>Mixture</i>	<i>Hybrid</i>
MC58	512	1024	512	1024	-	1024
NGH38	1024	2048	2048	4096	-	4096
BZ232	512	128	1024	16	-	-
MenA (F6124)	512	2048	2048	32	-	1024
MenC (C11)	>2048	n.d.	>2048	n.d.	-	n.d.
MenC (BZ133)	>4096	>8192	>4096	<16	-	2048

Hybrids of ORF46.1 and 919 were also constructed. Best results (four-fold higher titre) were achieved with 919 at the N-terminus.

- 15 Hybrids 919-519His, ORF97-225His and 225-ORF97His were also tested. These gave moderate ELISA fitres and bactericidal antibody responses.

As hybrids of two proteins A & B may be either NH₂-A-B-COOH or NH₂-B-A-COOH, the "reverse" hybrids with 287 at the N-terminus were also made, but using ΔG287. A panel of strains was used, including homologous strain 2996. FCA was used as adjuvant:

	287 & 919		287 & 953		287 & ORF46.1	
Strain	ΔG287-919	919-287	ΔG287-953	953-287	ΔG287-46.1	46.1-287
2996	128000	16000	65536	8192	16384	8192
BZ232	256	128	128	<4	<4	<4
1000	2048	<4	<4	<4	<4	<4
MC58	8192	1024	16384	1024	512	128
NGH38	32000	2048	>2048	4096	16384	4096
394/98	4096	32	256	128	128	16
MenA (F6124)	32000	2048	>2048	32	8192	1024
MenC (BZ133)	64000	>8192	>8192	<16	8192	2048

Better bactericidal titres are generally seen with 287 at the N-terminus.

When fused to protein 961 [NH₂-ΔG287-961-COOH – sequence shown above], the resulting

protein is insoluble and must be denatured and renatured for purification. Following renaturation, around 50% of the protein was found to remain insoluble. The soluble and

- 5 insoluble proteins were compared, and much better bactericidal titres were obtained with the soluble protein (FCA as adjuvant):

	2996	BZ232	MC58	NGH38	F6124	BZ133
Soluble	65536	128	4096	>2048	>2048	4096
Insoluble	8192	<4	<4	16	n.d.	n.d.

Titres with the insoluble form were, however, improved by using alum adjuvant instead:

Insoluble	32768	128	4096	>2048	>2048	2048
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- 10 961c was also used in hybrid proteins (see above). As 961 and its domain variants direct efficient expression, they are ideally suited as the N-terminal portion of a hybrid protein.

Example 23 – further hybrids

Further hybrid proteins of the invention are shown in the drawings and have the sequences set out below. These are advantageous when compared to the individual proteins:

15 **ORF46.1-741**
ATGTCAGATTGGCAAACGATTCTTTATCCGCAGGTTCTGACCGTCAGCATTCGAACCCGACGGAAATACCAC
CTATTCGGCAGCAGGGGGAACTTGCGAGGCCAGCGGCCATATCGGATTGGGAAAATACAAGCCATCAGTTGGGC
AACCTGATGATTCAACAGGCAGGCCATTAAAGGAAATATCGGCTACATTGTCCGTTTCCGATCACGGCACGAAGTC
CATTCCCCCTTCGACAACCATGCCTCACATTCCGATTCTGATGAAGCCGGTAGTCCCGTTGACGGATTAGCCTTTAC
CGCATCCATTGGGACGGATACGAACACCATCCCAGCCAGGGCTATGACGGGCCACAGGGCGGCGTATCCGCTCCC
AAAGGGCGAGGGATATATAACAGCTACGACATAAAAGCGTTGCCAAAATATCCGCTCAACCTGACCGACAACCGC
AGCACCGGACAACGGCTTGCGACCGTTCCACAATGCCGTAGTATGCTGACGCAAGGAGTAGGGGACGGATTCAA
CGGCCACCGATAACAGCCCCGAGCTGGACAGATCGGGCAATGCCCGAAGCCTCAACGGACTGCAGATACTGTT
AAAAACATCATCGGCGCGCAGGAGAAATTGTCGGCGAGGCGATGCCGTGCAGGGCATAAGCGAAGGCTAAACATT

GCTGTCATGCACGGCTTGGGTCTGCTTCCACCGAAAACAAGATGGCGCGCATCAACGATTGGCAGATATGGCGCAA
 CTCAAAGACTATGCCGCAGCAGCCATCCCGATTGGCAGTCCAAACCCCAATGCCGCACAAGGCATAGAAGCGTC
 5 AGCAATATCTTATGCCAGCCATCCCCATCAAAGGGATTGGAGCTGTCGGGAAATACGGCTTGGCGGCATCAGC
 GCACATCTATCAAGCGGTGCGAGATGGCGCGATCGCATTGCCGAAGGGAAATCCGTTCAAACTTGGAGCAGCGTTACGGCAA
 GATGCGGCATA CGC CAA AT ACCCGTCCCCTAACCGTCCCAGAAATGCCGTTCAAACTTGGAGCAGCGTTACGGCAA
 10 GAAAACATCACCTCTCAACCGTGCCCGTCAAACGGCAAAATGTCAAACCTGGCAGACCAACGCCACCCGAAAGACA
 GGC GTACCGTITGACGGTAAAGGGTTCCGAATT TGAGAAGCAGTGAAATATGATA CGGGATCCGGAGGGGTGGT
 GTCGCCGCCGACATCGGTGCGGGCTTGCCTGCGATGCACTAACCGCACCCTCGACCCATAAGACAAAGGTTGCAAGTCT
 TTGACGCTGGATCAGCTCGTCAAGGAAAAACGAGAAAATGAGCTGGCCGACAAGGTGCGGAAAAAAACTTATGAAAC
 15 GGTGACAGCCTCAATACGGCAATTGAGAAGCAGCAAGGTCAGCGTTTCGACTTATGCCCAAATCGAAGTGGAC
 GGGCAGCTCATTACCTTGGAGAGTGGAGACTTCAAGTATAACAAACAGGCCATTCCGCCCTAACCGCCCTTCAGACC
 GAGCAAATACAAGATTGGAGACTTCCGGAAAGATGGTTCGAAACGCCAGTTCAGAATCGGCACATAGCGGGCAA
 CATA CATCTTGACAAGCTTCCGAAGGCGCAGGGCAGATATCGGGGACGGCGTTCAGACGATGCCGAA
 GGAAAATGACCTACACCATAGATTGCGCCAAGCAGGGAAACGCCAAATGAAATGCCAGAACACTC
 20 AATGTCGACCTGGCCGCCGATATCAAGCCGGATGGAAAACGCCATGCCGTATCAGCGTTCCGTCTTACAAC
 CAAGCCGAGAAAGGCAGTTACTCCCTCGGTATCTTGGCGAAAAGCCCAGGAAGTTGCGGAGCGCGGAAGTGAAA
 ACCGTAACGGCATACGCCATATGCCCTGCCAGCAAGCAACTCGAGCACCACCAACTGA
 1 MSDLANDSFI RQVLDRHFE PDGKYHLFGS RGELAERSGH IGLKIQSHQ
 20 51 LGNLMIQQAA IKGNIGYIVR FSDHGHEVHS PFDNHASHSD SDEAGSPVDG
 101 FSLYRIHWDG YEHHPADGYD GPQGGGPAP KGARDIYSYD IKGVAQNIRL
 151 NLTDNRSTGQ RLADRFHNAG SMLTQGVGDG FKRATRYSPE LDRSGNAEEA
 201 FNGTADIVKN II GAAGEIVG AGDAVQGISE GSNIAVMHGL GLLSTENKMA
 25 251 RINDLADMAQ LKDYAAAIR DWAVQNPNAQ QGIEAVSNIF MAAPIKGIG
 301 AVR GKYG LGG ITAHPIKRSQ MGAI ALPKGK SAVSDNFADA AYAKYPSPYH
 351 SRNIRSNLEQ RYKGENITSS TVPPSNGKNV KLADQRHPKT GVPFDGKGFP
 401 NFEKHVKYDT GSGGGVAA D IGAGLADALT APLDHDKGL QSLTLQDSVR
 451 KNEKL LAAQ GAEKTYGN D SLNTGKLND KVS RFD FIRQ IEVDGQLITL
 501 ESGEFQVYKQ SHSAL TAFQT EQI QDSEHSG KMVA KQRFRI GDIAGEHTSF
 30 551 DKLPEGGRAT YRGTA FGSSD AGGKLTYTID F AAKQNGK I EHLKSP ELNV
 601 DLAAADIKPD GKRHA VISGS VLYNQAEKGS YSLGIFGGKA QEVAGSAEVK
 651 TVNGIRHIGL AAKQLEHHHH HH*

35 ORF46.1-961
 ATGTCAGATTGGCAAACGATTCTTTATCCGGCAGGTTCTGACCGTCAGCATTGCAACCCGACGGGAAATACAC
 CTATTCGGCAGCAGGGGGAACTTGGCAGGCCAGCGGCCATATCGGATTGGGAAATACAAAGCCATCAGTTGGC
 AACCTGATGATTCAACAGGCCG CATTAAAGGAAATATCGGCTACATTGTCGCTTCCGATCACGGGACGAAGTC
 CATTCCCCCTCGACAACCATGCCCTCACATTCCGATTCTGATGAAGCCGGTAGTCCCGTTGACGGATTAGCCTTAC
 40 CGC ATCCATTGGGACGGATACGACACCCATCCGCCGACGGCTATGACGGGCCACAGGGCGGGCTATCCGCTCC
 AAAGGCGCAGGGATATATACAGCTACGACATAAAGGCGTTGCCAAAATATCCGCTCAACTGACCGACAACCGC
 AGCACCGGACAACGGCTTGCGACCGTTCCACAATGCCGTAGTATGCTGACGCAAGGAGTAGGCGACGGATTCAA
 CGGCCACCCGATAACGCCCGAGCTGGACAGATCGGCCAATGCCGCGAAGCCTCAACGGCACTGCAGATATCGTT
 AAAACATCATCGGCCGCGCAGGAGAAAATTGCGGCCAGGGCGATGCCGTGCAGGGCATAAGCGAAGGCTCAA
 45 CATTGCTCATCGACGGCTTGGGCTGCTTCCACCGAAAACAGATGGCGCATACGATTGGCAGATATGGCGCAA
 CTCAAAGACTATGCCGCAGCAGCCATCCCGATTGGCAGTCCAAAACCCCAATGCCGACAAGGCATAGAAGCGTC
 AGCAATATCTTATGGCAGCCATCCCCATCAAAGGGATTGGAGCTGTCGGGAAATACGGCTTGGCGGCATCAGC
 GCACATCTATCAAGCGTCGAGATGGCGCGATCGCATTGCCGAAGGGAAATCCGCGTCAGCACAATTGCG
 GATGCCGCATACGCCAAATACCGTCCCCTAACATTCCGAAATATCGTTCAACTTGGAGCAGCGTTACGGCAA
 50 GAAAACATCACCTCTCAACCGTGCCCGTCAAACGGCAAAATGTCAAACTGGCAGACCAACGCCACCCGAAAGACA
 GGC GTACCGTTGACGGTAAAGGGTTCCGAATT TGAGAAGCAGTGAAATATGATA CGGGATCCGGAGGAGGAGGA
 GCCACAAACGACGACGATGTTAAAAGCTGCCACTGTGCCATTGCTGCTGCCATAACAAATGCCAAGAAATCAAC
 GGTTCAAGCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAGACGCAACTGCAGCGAT
 GTTGAAGCCGACGACTTAAAGGTCTGGGTCTGAAAAAAGCTGTGACTAACCTGACCAACCGTCAATGAAAACAAA
 55 CAAAACGTCGATGCCAAAGTAAAAGCTGCAAGATCTGAAATAGAAAAGTTAACACCAAGTTAGCAGACACTGATGCC
 GCTT TAGCAGATACTGATGCCCTCTGGATGCAACCACCAACGCCCTGAATAAAATGGGAGAAAATATAACGACATT
 GCTGAAGAGACTAAGACAAATATCGTAAAATTGATGAAAATTAGAAGCCGTTGCTGATACCGTCGACAAGCATGCC
 GAAGCATTCAACGATATGCCGATTGATGAAACCAACACTAACCGCAGACGAAGCCGTCAAACCGCCAATGAA
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 65 GCCGATCAGCAGATACTGCCCTGAAACGGTTGGGATAAAACAGTGTGCAAGACCTGCGCAAGAAACCGCCAAGGGCTTGCA
 GAACAAAGCGCGCTCCGGCTGTTCCAAACCTAACACGCTGGGCGTTCAATGTAACGGCTGCACTGGGGCTAC
 GGC ACTT CGTCCGGTCTTCCGCAAGCCTACCATGTCGGCGTCAATTACGAGTGGCTCGAGCACCACCAAC
 TGA

1 MSDLANDSF1 RQVLDROHFE PDGKYHLFGS RGELAERSGH IGLGKIQSHQ
 51 LGNLMIQQAA IKGNIGYIVR FSDHGHEVHS PFDNHASHSD SDEAGSPVDG
 5 101 FSLYRIHWDG YEHHPADGYD GPQGGGYPAP KGARDIYSYD IKGVAQNIRL
 151 NLTDNRSTGQ RLADRPHNAG SMLTQGVGDG FKTRATRYSPE LDRSGNAAEA
 201 FNGTADIVKN IIIGAAGEIVG AGDAVQGISE GSNIAVMHGL GLLSTENKMA
 251 RINDLADMAQ LKDYAAAIR DWAVQNPNAA QGIEAVSNIF MAAIPIKGIG
 301 AVRKYGLGG ITAHPIKRSQ MGAIALPKGK SAVSDNFADA AYAKYPSPYH
 351 SRNIRSNEQ RYKGKENITSS TVPPSNGKNV KLADQRHPKT GVPFDKGFP
 10 401 NFEKHVKYDT GSGGGGATND DDVKAATVA IAAAYNNQE INGFKAGETI
 451 YDIDEDGTIT KKDATAADVE ADDFKGLGLK KVVTNLTKTV NENKQNVDAK
 501 VKAAESEIEK LTTKLADTDA ALADTDAALD ATTNALNKLG ENITTFAEET
 551 KTNIVKIDEK LEAVADTVDK HAEAFNDIAD SLDETNKAD EAVKTANEAK
 601 QTAEETKQNV DAKVKAETA AGKAEAAAAGT ANTAADKAE A VAAKVTDIKA
 15 651 DIATNKDNIA KKANSADVYT REESDSKFVR IDGLNATTEK LDTRLASAEK
 701 SIADHDTRLN GLDKTVSDLR KETRQGLAEQ AALSGLFQPY NVGRFNVTAA
 751 VGGYKSESAC AIGTGFRFTE NFAAKAGVAV GTSSGSSAAY HVGVNEYLE
 801 HHHHHH*

20

ORF46.1-961c

ATGTCAGATTGGCAAACGATTCTTTATCCGGCAGGTTCTCGACCCTCAGCATTCGAACCCGACGGGAAATACAC
 CTATTGGCAGCAGGGGGAACTTGCAGCGCAGCGGCCATATCGGATTGGGAAAAATACAAAGCCATCAGTTGGC
 AACCTGATGATTCAACAGCGGCCATTAAAGGAAATATCGGCTACATTGTCGGCTTTCCGATCACGGCACGAAGTC
 25 CATTCCCCCTTCGACAACCATGCCACATTCCGATTCTGATGAAGCGGTAGTCCGTTGACGGATTAGCCTTTAC
 CGCATCCATTGGGACGGATACGAACACCATCCGGCAGCGCTATGACGGGCCACAGGGCGGGCTATCCGCTCCC
 AAAGGCGCAGGGATATATACAGCTACGACATAAAAGGCGTTGCCAAAATATCCGCTCACCTGACCGACAAACCGC
 AGCACCGGACAACGGCTTGGCAGCGTTCCACAATGCCGGTAGTATGCTGACGCAAGGAGTAGGGACGGATTCAAA
 CGGCCACCCGATACAGCCCCGAGCTGGACAGATGGCAGATGCCGCGAAGCCTCAACGGACTGCAGATATCGTT
 30 AAAAACATCATCGGCGGGCAGGAGAAATTGTCGGCGCAGGCATGCCGTTCCACCGAAAACAGATGGCGCAGTCAACGATTGGCAGATATGGCGAA
 GCTGTCATGCACGGCTGGGTCTGCTTCCACCGAAAACAGATGGCGCAGTCCAAAACCCAAATGCCGACAAGGCATAGAACCGTC
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 AGCAATATCTTATGCCAGCCATCCCATCAAAGGATTGGAGCTGTCGGGAAAATACGCTTGGGGCGCATCACG
 GCACATCCTATCAAGGGTCGAGTGGCGCAGTCGCTTGGGAAATCCGCTCAGCGACAATTGGC
 35 35 GATGCGGCATACGCAAATACCGCTCCATTACCATCCGAAATATCGTCAAACCTGGAGCAGCGCTTACGGCAA
 GAAAACATCACCTCTAACCGTGGCGCGTCACCGAAAATATGTCAGGAGCAGCACGTAAGGAAATATGATACGGGATCCGGAGGAGGA
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 40 GGTTCAAAGCTGGAGAGACCATCAGACATTGATGAAGACGGCACAACTTACCAAAACCGTCAATGAAAACAAA
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 CAAAACGTCGATGCCAAGTAAAAGCTGAGAATCTGAAATAGAAAAGTTAACACCAAGTTACGAGACACTGATGCC
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 45 GCTGAAGAGACTAAGACAAAATATCGTAAAATATGATGAAAATTAGAAGCCGTGGCTGATACCGTGCACAAAGCATGCC
 GAAGCATTCAACGATATGCCGATTCTGGATGAAACCAACACTAAGGCAGACGAAGCCGTAAAACCGCAATGAA
 50 GCCAAACAGACGGCGAAGAAACCAAACAAACCGTCAAGTAAAAGCTGCAAGAAACTGCCAGCAGGCAAAGCC
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 GAACAAGCCCGCTCCGGTCTGTTCAACCTTACAACGTGGGCTCGAGCACCACCAACCACTG

1 MSDLANDSF1 RQVLDROHFE PDGKYHLFGS RGELAERSGH IGLGKIQSHQ
 51 LGNLMIQQAA IKGNIGYIVR FSDHGHEVHS PFDNHASHSD SDEAGSPVDG
 55 101 FSLYRIHWDG YEHHPADGYD GPQGGGYPAP KGARDIYSYD IKGVAQNIRL
 151 NLTDNRSTGQ RLADRPHNAG SMLTQGVGDG FKTRATRYSPE LDRSGNAAEA
 201 FNGTADIVKN IIIGAAGEIVG AGDAVQGISE GSNIAVMHGL GLLSTENKMA
 251 RINDLADMAQ LKDYAAAIR DWAVQNPNAA QGIEAVSNIF MAAIPIKGIG
 301 AVRKYGLGG ITAHPIKRSQ MGAIALPKGK SAVSDNFADA AYAKYPSPYH
 351 SRNIRSNEQ RYKGKENITSS TVPPSNGKNV KLADQRHPKT GVPFDKGFP
 60 401 NFEKHVKYDT GSGGGGATND DDVKAATVA IAAAYNNQE INGFKAGETI
 451 YDIDEDGTIT KKDATAADVE ADDFKGLGLK KVVTNLTKTV NENKQNVDAK
 501 VKAAESEIEK LTTKLADTDA ALADTDAALD ATTNALNKLG ENITTFAEET
 551 KTNIVKIDEK LEAVADTVDK HAEAFNDIAD SLDETNKAD EAVKTANEAK
 601 QTAEETKQNV DAKVKAETA AGKAEAAAAGT ANTAADKAE A VAAKVTDIKA
 65 651 DIATNKDNIA KKANSADVYT REESDSKFVR IDGLNATTEK LDTRLASAEK
 701 SIADHDTRLN GLDKTVSDLR KETRQGLAEQ AALSGLFQPY NVGLEHHHHH
 751 H*

961-ORF46.1

5 ATGGCCACAAACGACGACGATGTTAAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCTACAACAATGGCAAGAAATC
 AACGGTTCAAAGCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAAGACGCAACTGCAGCC
 GATGTTGAAGCCGACGACTTTAAAGGTCTGGTCTGAAAAAAGTCGTGACTAACCTGACCAAAACCGTCAATGAAAAC
 AAACAAAACGTCGATGCCAAAGTAAAAGCTGAGAATCTGAAATAGAAAAGTTAACCAACCAAGTTAGCAGACACTGAT
 GCCGCTTTAGCAGATACTGATGCCGCTCTGGATGCAACCACCAACGCCCTGATAATTGGGAGAAAATATAACGACA
 TTTGCTGAAGAGACTAAGACAAATATCGTAAAAAATTGATGAAAAAATTAGAAGGCCGTTGGCTGATACCGTCGACAAGCAT
 10 GCCGAAAGCATTCAACGATATGCCGATTCTGGATGAAACCAACACTAACGGCTTGACACACGCTGGCTTCTGCTGAAAAC
 GAAGCCAACAGACGGCGAAGAAACCAACAAAACGTCGATGCCAAAGTAAAAGCTGCCAGAAAATGCAAGCAGGCCAA
 GCCGAAGCTGCCGCTGCCACAGCTAACACTGCAAGCCGAAAGCTGCTGCAAAAGTTACCGACATCAA
 GCTGATATCGCTACGAAACAAAGATAATATTGCTAAAAAAGCAACAGTGCCTGACCGAGAAGAGTCTGAC
 AGCAAAATTGTCAGAATTGATGGCTGTAACGCTACTACCGAAAATTGACACACGCTTGGCTTCTGCTGAAAATCC
 15 ATTGGCGATCACGATACTGCCCTGAAACGGTTGGATAAAACAGTGTCAAGCCTGCGAAAGAAACCGCAAGGCCCTT
 GCAGAACAGCCGCTCTCCGGCTGTTCCAACCTTACAACGTGGGTCGGTTCAATGTAACGGCTGCAGTCGGCGC
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 GTCCGGACTTCGTCGGTTCTCCGCTACCATGTCGGCTCAATTACGAGTGGGATCCGGAGGAGGAGGATCA
 GATTTGGCAAACGATTCTTTATCCGGCAGGTCTCGACCGTCAGCATTGAAACCCGACGGAAATACCAACCTATT
 20 GGCAGCAGGGGGAACTTGCCGAGCGCAGCGGCCATATGGATTGGAAAAATACAAAGCCATCAGTTGGCAACCTG
 ATGATTCAACAGGGGGCATTAAAGGAAATATGGCTACATTGTCGCTTTCCGATCACGGGACGAAGTCCATT
 CCCCTCGACAACCATTGCCACATTCCGATTCTGATGAAGCCGGTAGTCCCGTTGACGGATTAGCCTTTACCGCATC
 CATTGGGACGGATACGAACACCATTCCGCCACGGCTATGACGGGCCACAGGGCGGGCTATCCGCTCCCAAAGGC
 25 GCGAGGGATATATACAGCTACGACATAAAAGCGTTGCCAAAATATCCGCTCAACCTGACCACCGCAGCACC
 GGACAACGGCTTGCCGACCCTTCCACAATGCCGGTAGTATGCTGACGCAAGGAGTAGGCGACGGATTCAAACCGGCC
 ACCCGATAAGCCCCGAGCTGGACAGATGGCAATGCCGGGAAGCCTTCAACGGCACTGCAAGATATGTTAAAAC
 ATCATCGGCGGGCAGGAGAAATTGTCGGCGCAGGCGATGCCGTGCAAGGGCATAAGGCAAGGCTAAACATTGCTGTC
 ATGCAACGGCTTGGGCTGCTTCCACCGAAAACAAGATGCCGCATCAACGATTGGCAGATATGGCGCAACTCAA
 30 GACTATGCGCAGCAGCCATCCGCAATTGGGAGTCCAAAACCGGATGGAGCTGTTGGGAAATACGGCTTGGGCGCAT
 ATCTTATGGCAGCCATCCCACCAAGGGGATGGAGCTGTTGGGAAATACGGCTTGGGCGCATCACGGCACAT
 CCTATCAAGCGGTCGAGATGGCGCATGCGATTGCCGAAAGGGGAAATCCGCGTCAACGGCAACTGGGAGGCT
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 ATCACCTCTCAACCGTGCCGCCGTCAAACGGAAAATGTCAAACTGGCAGACCAACGCCACCGAAGACAGCGTA
 CCGTTGACGGTAAAGGTTCCGAAATTGAGAACGTGAAATATGATACGCTGAGCACCACACCAC
 35 TGA

1	MATNDDDVKK AATVAIAAAAY NNGQEINGFK AGETIYDIDE DGTITKDKAT
51	AADVEADDK GLGLKKVVTN LTKTVNENKQ NVDAKVKAEE SEIEKLTTKL
101	ADTDAAALADT DAALDATTNA LNKLGENITT FAEETKTNIV KIDEKLEAVA
151	DTVDKHAEAF NDIADSLDET NTKADEAVKT ANEAKQTAEE TKQNVDAKVK
201	AAETAAGKAE AAACTANTAA DKAEEAVAAKV TDIKADIATN KDNIAKKANS
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801	HHHHHH*

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-28-

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1351	KAFLFATAGV ERDLNGRDYT VTGGFTGATA ATGKTGARNM PHTRLVAGLG
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	751	H*

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961c-983

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 751 KGKFPNFEKH VKYDT*

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 851 NNTYTGKTII EGGSLVLYGN NKSMDRVEETK GALIYNGAAS GGSLNSDGIV
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 1351 KTGARNMPHT RLVAGLGADV EPGNGWNGLA RYSYAGSKQY GNHSGRVGVG
 1401 YRF*

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention. For instance, the use of proteins from other strains is envisaged [e.g. see WO00/66741 for polymorphic sequences for ORF4, ORF40, ORF46, 225, 235, 287, 519, 726, 919 and 953].

5

EXPERIMENTAL DETAILS

Cloning strategy and oligonucleotide design

Genes coding for antigens of interest were amplified by PCR, using oligonucleotides designed on the basis of the genomic sequence of *N. meningitidis* B MC58. Genomic DNA 10 from strain 2996 was always used as a template in PCR reactions, unless otherwise specified, and the amplified fragments were cloned in the expression vector pET21b+ (Novagen) to express the protein as C-terminal His-tagged product, or in pET-24b+(Novagen) to express the protein in 'untagged' form (e.g. ΔG 287K).

Where a protein was expressed without a fusion partner and with its own leader peptide (if 15 present), amplification of the open reading frame (ATG to STOP codons) was performed.

Where a protein was expressed in 'untagged' form, the leader peptide was omitted by designing the 5'-end amplification primer downstream from the predicted leader sequence.

The melting temperature of the primers used in PCR depended on the number and type of hybridising nucleotides in the whole primer, and was determined using the formulae:

$$T_{m1} = 4(G+C) + 2(A+T) \quad (\text{tail excluded})$$

$$T_{m2} = 64.9 + 0.41(\% \text{ GC}) - 600/N \quad (\text{whole primer})$$

The melting temperatures of the selected oligonucleotides were usually 65-70°C for the whole oligo and 50-60°C for the hybridising region alone.

Oligonucleotides were synthesised using a Perkin Elmer 394 DNA/RNA Synthesizer, eluted 25 from the columns in 2.0ml NH₄OH, and deprotected by 5 hours incubation at 56°C. The oligos were precipitated by addition of 0.3M Na-Acetate and 2 volumes ethanol. The samples were centrifuged and the pellets resuspended in water.

		Sequences	Restriction site
fu (961)-	Fwd	CGCGGGATCC -GGAGGGGGTGGTGTGTCG	BamHI

741(MC58)-His			
	Rev	CCCGCTCGAG-TTGCTTGGCGGCAAGGC	XhoI
fu (961)-983-His	Fwd	CGCGGATCC - GGCGGAGGCAGGCACTT	BamHI
	Rev	CCCGCTCGAG-GAACCGGTAGCCTACG	XhoI
fu (961)- Orf46.1- His	Fwd	CGCGGATCCGGTGGTGGTGGT- TCAGATTGGCAAACGATT	BamHI
	Rev	CCCGCTCGAG-CGTATCATATTCACGTGC	XhoI
fu (961 c-L)- 741(MC58)	Fwd	CGCGGATCC -GGAGGGGGTGGTGTGCG	BamHI
	Rev	CCCGCTCGAG-TTATTGCTTGGCGGCAAG	XhoI
fu (961c-L)-983	Fwd	CGCGGATCC - GGCGGAGGCAGGCACTT	BamHI
	Rev	CCCGCTCGAG-TCAGAACCGGTAGCCTAC	XhoI
fu (961c-L)- Orf46.1	Fwd	CGCGGATCCGGTGGTGGTGGT- TCAGATTGGCAAACGATT	BamHI
	Rev	CCCGCTCGAG-TTACGTATCATATTCACGTGC	XhoI
fu-(ΔG287)-919- His	Fwd	CGCGGATCCGGTGGTGGTGGT- CAAAGCAAGAGCATCCAAACC	BamHI
	Rev	CCCAAGCTT-TTCGGCGGTATTGGGCTTC	HindIII
fu-(ΔG287)-953- His	Fwd	CGCGGATCCGGTGGTGGTGGT- GCCACCTACAAAGTGGAC	BamHI
	Rev	GCCCAAGCTT-TTGTTGGCTGCCTCGAT	HindIII
fu-(ΔG287)-961- His	Fwd	CGCGGATCCGGTGGTGGTGGT-ACAAGCGACGACG	BamHI
	Rev	GCCCAAGCTT-CCACTCGTAATTGACGCC	HindIII
fu-(ΔG287)- Orf46.1-His	Fwd	CGCGGATCCGGTGGTGGTGGT- TCAGATTGGCAAACGATT	BamHI
	Rev	CCCAAGCTT-CGTATCATATTCACGTGC	HindIII
fu-(ΔG287-919)- Orf46.1-His	Fwd	CCCAAGCTTGGTGGTGGTGGTGGT- TCAGATTGGCAAACGATT	HindIII
	Rev	CCCGCTCGAG-CGTATCATATTCACGTGC	XhoI
fu-(ΔG287- Orf46.1)-919-His	Fwd	CCCAAGCTTGGTGGTGGTGGTGGT- CAAAGCAAGAGCATCCAAACC	HindIII
	Rev	CCCGCTCGAG-CGGGCGGTATTGGGCTTC	XhoI
fu ΔG287(394.98)-	Fwd	CGCGGATCCCGTAGC-CCCGATGTTAAATCGGC	NheI
	Rev	CGGGGATCC-ATCCTGCTTTTTGCCGG	BamHI
fu Orf1-(Orf46.1)- His	Fwd	CGCGGATCCCGTAGC-GGACACACTTATTCGGCAGC	NheI
	Rev	CGCGGATCC-CCAGCGGTAGCCTAATTGAT	
fu (Orf1)-Orf46.1- His	Fwd	CGCGGATCCGGTGGTGGTGGT- TCAGATTGGCAAACGATT	BamHI
	Rev	CCCAAGCTT-CGTATCATATTCACGTGC	HindIII
fu (919)-Orf46.1- His	Fwd1	CGGGCGTCGACGGTGGCGGAGGCAGTGGATCCTCAG	SalI
	Fwd2	GGAGGGCACTGGATCCTCAGATTGGCAAACGATT	
	Rev	CCCGCTCGAG-CGTATCATATTCACGTGC	XhoI
Fu (orf46)-287-His	Fwd	CGGGGATCCGGGGCGGGTGGCG	BamHI
	Rev	CCCAAGCTTATCCTGCTTTTTGCCGGC	HindIII
Fu (orf46)-919-His	Fwd	CGCGGATCCGGTGGTGGTCAAAGCAAGAGCATCCA AACC	BamHI
	Rev	CCCAAGCTTGGCGGTATTGGGCTTC	HindIII

Fu (orf46-919)-287-His	Fwd	CCCCAAGCTTGGGGCGCGGTGGCG	HindIII
	Rev	CCCGCTCGAGATCCTGCTTTTGCCGGC	XhoI
Fu (orf46-287)-919-His	Fwd	CCCAAGCTTGGTGGTGGTGGTCAAAGCAAGAGCAT	HindIII
	Rev	CCCGCTCGAGCGGGCGGTATTGGCTT	XhoI
(ΔG741)-961c-His	Fwd1	GGAGGCACTGGATCCGCAGCCACAAACGACGACGA	XhoI
	Fwd2	GCGGCCCTCGAG-GGTGGCGGAGGCAGTGGATCCGCAG	
	Rev	CCCGCTCGAG-ACCCAGCTTGTAAAGGTTG	XhoI
(ΔG741)-961-His	Fwd1	GGAGGCACTGGATCCGCAGCCACAAACGACGACGA	XhoI
	Fwd2	GCGGCCCTCGAG-GGTGGCGGAGGCAGTGGATCCGCAG	
	Rev	CCCGCTCGAG-CCACTCGTAATTGACGCC	XhoI
(ΔG741)-983-His	Fwd	GCGGCCCTCGAG-GGATCCGGCGGAGGCAGTCGCG	XhoI
	Rev	CCCGCTCGAG-GAACCGGTAGCCTACG	XhoI
(ΔG741)-orf46.1-His	Fwd1	GGAGGCACTGGATCCTCAGATTGGCAAACGATT	SalI
	Fwd2	GCGGCCTCGACGGTGGCGGAGGCAGTGGATCCTCAGA	
	Rev	CCCGCTCGAG-CGTATCATATTACGTGC	XhoI
(ΔG983)-741(MC58)-His	Fwd	GCGGCCCTCGAG-GGATCCGGAGGGGGTGGTGTGCC	XhoI
	Rev	CCCGCTCGAG-TTGCTTGGCGGCAAG	XhoI
(ΔG983)-961c-His	Fwd1	GGAGGCACTGGATCCGCAGCCACAAACGACGACGA	XhoI
	Fwd2	GCGGCCCTCGAG-GGTGGCGGAGGCAGTGGATCCGCAG	
	Rev	CCCGCTCGAG-ACCCAGCTTGTAAAGGTTG	XhoI
(ΔG983)-961-His	Fwd1	GGAGGCACTGGATCCGCAGCCACAAACGACGACGA	XhoI
	Fwd2	GCGGCCCTCGAG-GGTGGCGGAGGCAGTGGATCCGCAG	
	Rev	CCCGCTCGAG-CCACTCGTAATTGACGCC	XhoI
(ΔG983)-Orf46.1-His	Fwd1	GGAGGCACTGGATCCTCAGATTGGCAAACGATT	SalI
	Fwd2	GCGGCCTCGACGGTGGCGGAGGCAGTGGATCCTCAGA	
	Rev	CCCGCTCGAG-CGTATCATATTACGTGC	XhoI

* This primer was used as a Reverse primer for all the C terminal fusions of 287 to the His-tag.

^b Forward primers used in combination with the 287-His Reverse primer.

NB – All PCR reactions use strain 2996 unless otherwise specified (e.g. strain MC58)

In all constructs starting with an ATG not followed by a unique *Nhe*I site, the ATG codon is part of the *Nde*I site used for cloning. The constructs made using *Nhe*I as a cloning site at the 5' end (e.g. all those containing 287 at the N-terminus) have two additional codons (GCT AGC) fused to the coding sequence of the antigen.

Preparation of chromosomal DNA templates

N.meningitidis strains 2996, MC58, 394.98, 1000 and BZ232 (and others) were grown to exponential phase in 100ml of GC medium, harvested by centrifugation, and resuspended in 5ml buffer (20% w/v sucrose, 50mM Tris-HCl, 50mM EDTA, pH8). After 10 minutes incubation on ice, the bacteria were lysed by adding 10ml of lysis solution (50mM NaCl, 1% Na-Sarkosyl, 50μg/ml Proteinase K), and the suspension incubated at 37°C for 2 hours. Two

phenol extractions (equilibrated to pH 8) and one CHCl₃/isoamylalcohol (24:1) extraction were performed. DNA was precipitated by addition of 0.3M sodium acetate and 2 volumes of ethanol, and collected by centrifugation. The pellet was washed once with 70%(v/v) ethanol and redissolved in 4.0ml TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0). The 5 DNA concentration was measured by reading OD₂₆₀.

PCR Amplification

The standard PCR protocol was as follows: 200ng of genomic DNA from 2996, MC581000, or BZ232 strains or 10ng of plasmid DNA preparation of recombinant clones were used as template in the presence of 40μM of each oligonucleotide primer, 400-800 μM dNTPs 10 solution, 1x PCR buffer (including 1.5mM MgCl₂), 2.5 units *TaqI* DNA polymerase (using Perkin-Elmer AmpliTaqQ, Boerhingher Mannheim Expand™ Long Template).

After a preliminary 3 minute incubation of the whole mix at 95°C, each sample underwent a two-step amplification: the first 5 cycles were performed using the hybridisation temperature that excluded the restriction enzyme tail of the primer (T_{m1}). This was followed by 30 cycles 15 according to the hybridisation temperature calculated for the whole length oligos (T_{m2}). Elongation times, performed at 68°C or 72°C, varied according to the length of the Orf to be amplified. In the case of Orf1 the elongation time, starting from 3 minutes, was increased by 15 seconds each cycle. The cycles were completed with a 10 minute extension step at 72°C.

The amplified DNA was either loaded directly on a 1% agarose gel. The DNA fragment 20 corresponding to the band of correct size was purified from the gel using the Qiagen Gel Extraction Kit, following the manufacturer's protocol.

Digestion of PCR fragments and of the cloning vectors

The purified DNA corresponding to the amplified fragment was digested with the appropriate restriction enzymes for cloning into pET-21b+, pET22b+ or pET-24b+. Digested 25 fragments were purified using the QIAquick PCR purification kit (following the manufacturer's instructions) and eluted with either H₂O or 10mM Tris, pH 8.5. Plasmid vectors were digested with the appropriate restriction enzymes, loaded onto a 1.0% agarose gel and the band corresponding to the digested vector purified using the Qiagen QIAquick Gel Extraction Kit.

Cloning

The fragments corresponding to each gene, previously digested and purified, were ligated into pET21b+, pET22b+ or pET-24b+. A molar ratio of 3:1 fragment/vector was used with T4 DNA ligase in the ligation buffer supplied by the manufacturer.

- 5 Recombinant plasmid was transformed into competent *E.coli* DH5 or HB101 by incubating the ligase reaction solution and bacteria for 40 minutes on ice, then at 37°C for 3 minutes. This was followed by the addition of 800μl LB broth and incubation at 37°C for 20 minutes. The cells were centrifuged at maximum speed in an Eppendorf microfuge, resuspended in approximately 200μl of the supernatant and plated onto LB ampicillin (100mg/ml) agar.
- 10 Screening for recombinant clones was performed by growing randomly selected colonies overnight at 37°C in 4.0ml of LB broth + 100μg/ml ampicillin. Cells were pelleted and plasmid DNA extracted using the Qiagen QIAprep Spin Miniprep Kit, following the manufacturer's instructions. Approximately 1μg of each individual miniprep was digested with the appropriate restriction enzymes and the digest loaded onto a 1-1.5% agarose gel
- 15 (depending on the expected insert size), in parallel with the molecular weight marker (1kb DNA Ladder, GIBCO). Positive clones were selected on the basis of the size of insert.

Expression

- After cloning each gene into the expression vector, recombinant plasmids were transformed into *E.coli* strains suitable for expression of the recombinant protein. 1μl of each construct was used to transform *E.coli* BL21-DE3 as described above. Single recombinant colonies were inoculated into 2ml LB+Amp (100μg/ml), incubated at 37°C overnight, then diluted 1:30 in 20ml of LB+Amp (100μg/ml) in 100ml flasks, to give an OD₆₀₀ between 0.1 and 0.2. The flasks were incubated at 30°C or at 37°C in a gyratory water bath shaker until OD₆₀₀ indicated exponential growth suitable for induction of expression (0.4-0.8 OD). Protein expression was induced by addition of 1.0mM IPTG. After 3 hours incubation at 30°C or 37°C the OD₆₀₀ was measured and expression examined. 1.0ml of each sample was centrifuged in a microfuge, the pellet resuspended in PBS and analysed by SDS-PAGE and Coomassie Blue staining.

Purification of His-tagged proteins

- 30 Various forms of 287 were cloned from strains 2996 and MC58. They were constructed with a C-terminus His-tagged fusion and included a mature form (aa 18-427), constructs with

deletions ($\Delta 1$, $\Delta 2$, $\Delta 3$ and $\Delta 4$) and clones composed of either B or C domains. For each clone purified as a His-fusion, a single colony was streaked and grown overnight at 37°C on a LB/Amp (100 µg/ml) agar plate. An isolated colony from this plate was inoculated into 20ml of LB/Amp (100 µg/ml) liquid medium and grown overnight at 37°C with shaking.

5 The overnight culture was diluted 1:30 into 1.0 L LB/Amp (100 µg/ml) liquid medium and allowed to grow at the optimal temperature (30 or 37°C) until the OD₅₅₀ reached 0.6-0.8. Expression of recombinant protein was induced by addition of IPTG (final concentration 1.0mM) and the culture incubated for a further 3 hours. Bacteria were harvested by centrifugation at 8000g for 15 min at 4°C. The bacterial pellet was resuspended in 7.5 ml of

10 either (i) cold buffer A (300 mM NaCl, 50 mM phosphate buffer, 10 mM imidazole, pH 8.0) for soluble proteins or (ii) buffer B (10mM Tris-HCl, 100 mM phosphate buffer, pH 8.8 and, optionally, 8M urea) for insoluble proteins. Proteins purified in a soluble form included 287-His, $\Delta 1$, $\Delta 2$, $\Delta 3$ and $\Delta 4$ 287-His, $\Delta 4$ 287MC58-His, 287c-His and 287cMC58-His. Protein 287bMC58-His was insoluble and purified accordingly. Cells were disrupted by

15 sonication on ice four times for 30 sec at 40W using a Branson sonifier 450 and centrifuged at 13000xg for 30 min at 4°C. For insoluble proteins, pellets were resuspended in 2.0 ml buffer C (6 M guanidine hydrochloride, 100 mM phosphate buffer, 10 mM Tris- HCl, pH 7.5 and treated with 10 passes of a Dounce homogenizer. The homogenate was centrifuged at 13000g for 30 min and the supernatant retained. Supernatants for both soluble and insoluble

20 preparations were mixed with 150µl Ni²⁺-resin (previously equilibrated with either buffer A or buffer B, as appropriate) and incubated at room temperature with gentle agitation for 30 min. The resin was Chelating Sepharose Fast Flow (Pharmacia), prepared according to the manufacturer's protocol. The batch-wise preparation was centrifuged at 700g for 5 min at 4°C and the supernatant discarded. The resin was washed twice (batch-wise) with 10ml

25 buffer A or B for 10 min, resuspended in 1.0 ml buffer A or B and loaded onto a disposable column. The resin continued to be washed with either (i) buffer A at 4°C or (ii) buffer B at room temperature, until the OD₂₈₀ of the flow-through reached 0.02-0.01. The resin was further washed with either (i) cold buffer C (300mM NaCl, 50mM phosphate buffer, 20mM imidazole, pH 8.0) or (ii) buffer D (10mM Tris-HCl, 100mM phosphate buffer, pH 6.3 and, optionally, 8M urea) until OD₂₈₀ of the flow-through reached 0.02-0.01. The His-fusion

30 protein was eluted by addition of 700µl of either (i) cold elution buffer A (300 mM NaCl, 50mM phosphate buffer, 250 mM imidazole, pH 8.0) or (ii) elution buffer B (10 mM Tris-HCl, 100 mM phosphate buffer, pH 4.5 and, optionally, 8M urea) and fractions

collected until the OD₂₈₀ indicated all the recombinant protein was obtained. 20µl aliquots of each elution fraction were analysed by SDS-PAGE. Protein concentrations were estimated using the Bradford assay.

Renaturation of denatured His-fusion proteins.

- 5 Denaturation was required to solubilize 287bMC8, so a renaturation step was employed prior to immunisation. Glycerol was added to the denatured fractions obtained above to give a final concentration of 10% v/v. The proteins were diluted to 200 µg/ml using dialysis buffer I (10% v/v glycerol, 0.5M arginine, 50 mM phosphate buffer, 5.0 mM reduced glutathione, 0.5 mM oxidised glutathione, 2.0M urea, pH 8.8) and dialysed against the same buffer for
10 12-14 hours at 4°C. Further dialysis was performed with buffer II (10% v/v glycerol, 0.5M arginine, 50mM phosphate buffer, 5.0mM reduced glutathione, 0.5mM oxidised glutathione, pH 8.8) for 12-14 hours at 4°C. Protein concentration was estimated using the formula:

$$\text{Protein (mg/ml)} = (1.55 \times OD_{280}) - (0.76 \times OD_{260})$$

Immunization

- 15 Balb/C mice were immunized with antigens on days 0, 21 and 35 and sera analyzed at day 49.

Sera analysis – ELISA

- The acapsulated MenB M7 and the capsulated strains were plated on chocolate agar plates and incubated overnight at 37°C with 5% CO₂. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into Mueller-Hinton Broth (Difco) containing 0.25% glucose. Bacterial growth was monitored every 30 minutes by following OD₆₂₀. The bacteria were let to grow until the OD reached the value of 0.4-0.5. The culture was centrifuged for 10 minutes at 4000rpm. The supernatant was discarded and bacteria were washed twice with PBS, resuspended in PBS containing 0.025% formaldehyde, and incubated for 1 hour at 37°C and then overnight at 4°C with stirring. 100µl bacterial cells 25 were added to each well of a 96 well Greiner plate and incubated overnight at 4°C. The wells were then washed three times with PBT washing buffer (0.1% Tween-20 in PBS). 200µl of saturation buffer (2.7% polyvinylpyrrolidone 10 in water) was added to each well and the plates incubated for 2 hours at 37°C. Wells were washed three times with PBT. 200µl of diluted sera (Dilution buffer: 1% BSA, 0.1% Tween-20, 0.1% NaN₃ in PBS) were added to 30 each well and the plates incubated for 2 hours at 37°C. Wells were washed three times with PBT. 100µl of HRP-conjugated rabbit anti-mouse (Dako) serum diluted 1:2000 in dilution buffer were added to each well and the plates were incubated for 90 minutes at 37°C. Wells

were washed three times with PBT buffer. 100 μ l of substrate buffer for HRP (25ml of citrate buffer pH5, 10mg of O-phenyldiamine and 10 μ l of H₂O₂) were added to each well and the plates were left at room temperature for 20 minutes. 100 μ l 12.5% H₂SO₄ was added to each well and OD₄₉₀ was followed. The ELISA titers were calculated arbitrarily as the dilution of sera which gave an OD₄₉₀ value of 0.4 above the level of preimmune sera. The ELISA was considered positive when the dilution of sera with OD₄₉₀ of 0.4 was higher than 1:400.

Sera analysis – FACS Scan bacteria binding assay

The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37°C with 5% CO₂. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into 4 tubes containing 8ml each Mueller-Hinton Broth (Difco) containing 0.25% glucose. Bacterial growth was monitored every 30 minutes by following OD₆₂₀. The bacteria were let to grow until the OD reached the value of 0.35-0.5. The culture was centrifuged for 10 minutes at 4000rpm. The supernatant was discarded and the pellet was resuspended in blocking buffer (1% BSA in PBS, 0.4% NaN₃) and centrifuged for 5 minutes at 4000rpm. Cells were resuspended in blocking buffer to reach OD₆₂₀ of 0.05. 100 μ l bacterial cells were added to each well of a Costar 96 well plate. 100 μ l of diluted (1:100, 1:200, 1:400) sera (in blocking buffer) were added to each well and plates incubated for 2 hours at 4°C. Cells were centrifuged for 5 minutes at 4000rpm, the supernatant aspirated and cells washed by addition of 200 μ l/well of blocking buffer in each well. 100 μ l of R-Phicoerytrin conjugated F(ab)₂ goat anti-mouse, diluted 1:100, was added to each well and plates incubated for 1 hour at 4°C. Cells were spun down by centrifugation at 4000rpm for 5 minutes and washed by addition of 200 μ l/well of blocking buffer. The supernatant was aspirated and cells resuspended in 200 μ l/well of PBS, 0.25% formaldehyde. Samples were transferred to FACScan tubes and read. The condition for FACScan (Laser Power 15mW) setting were: FL2 on; FSC-H threshold:92; FSC PMT Voltage: E 01; SSC PMT: 474; Amp. Gains 6.1; FL-2 PMT: 586; compensation values: 0.

Sera analysis – bactericidal assay

N. meningitidis strain 2996 was grown overnight at 37°C on chocolate agar plates (starting from a frozen stock) with 5% CO₂. Colonies were collected and used to inoculate 7ml Mueller-Hinton broth, containing 0.25% glucose to reach an OD₆₂₀ of 0.05-0.08. The culture was incubated for approximately 1.5 hours at 37 degrees with shacking until the OD₆₂₀ reached the value of 0.23-0.24. Bacteria were diluted in 50mM Phosphate buffer pH 7.2 containing 10mM MgCl₂, 10mM CaCl₂ and 0.5% (w/v) BSA (assay buffer) at the working dilution of 10⁵ CFU/ml. The total volume of the final reaction mixture was 50 μ l with 25 μ l

of serial two fold dilution of test serum, 12.5 µl of bacteria at the working dilution, 12.5 µl of baby rabbit complement (final concentration 25%).

Controls included bacteria incubated with complement serum, immune sera incubated with bacteria and with complement inactivated by heating at 56°C for 30'. Immediately after the 5 addition of the baby rabbit complement, 10µl of the controls were plated on Mueller-Hinton agar plates using the tilt method (time 0). The 96-wells plate was incubated for 1 hour at 37°C with rotation. 7µl of each sample were plated on Mueller-Hinton agar plates as spots, whereas 10µl of the controls were plated on Mueller-Hinton agar plates using the tilt method (time 1). Agar plates were incubated for 18 hours at 37 degrees and the colonies 10 corresponding to time 0 and time 1 were counted.

Sera analysis – western blots

Purified proteins (500ng/lane), outer membrane vesicles (5µg) and total cell extracts (25µg) derived from MenB strain 2996 were loaded onto a 12% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. The transfer was performed for 2 hours at 150mA 15 at 4°C, using transfer buffer (0.3% Tris base, 1.44% glycine, 20% (v/v) methanol). The membrane was saturated by overnight incubation at 4°C in saturation buffer (10% skimmed milk, 0.1% Triton X100 in PBS). The membrane was washed twice with washing buffer (3% skimmed milk, 0.1% Triton X100 in PBS) and incubated for 2 hours at 37°C with mice sera diluted 1:200 in washing buffer. The membrane was washed twice and incubated for 90 20 minutes with a 1:2000 dilution of horseradish peroxidase labelled anti-mouse Ig. The membrane was washed twice with 0.1% Triton X100 in PBS and developed with the Opti-4CN Substrate Kit (Bio-Rad). The reaction was stopped by adding water.

The OMVs were prepared as follows: *N. meningitidis* strain 2996 was grown overnight at 37 degrees with 5% CO₂ on 5 GC plates, harvested with a loop and resuspended in 10 ml of 25 20mM Tris-HCl pH 7.5, 2 mM EDTA. Heat inactivation was performed at 56°C for 45 minutes and the bacteria disrupted by sonication for 5 minutes on ice (50% duty cycle, 50% output , Branson sonifier 3 mm microtip). Unbroken cells were removed by centrifugation at 5000g for 10 minutes, the supernatant containing the total cell envelope fraction recovered and further centrifuged overnight at 50000g at the temperature of 4°C . The pellet containing 30 the membranes was resuspended in 2% sarkosyl, 20mM Tris-HCl pH 7.5, 2 mM EDTA and incubated at room temperature for 20 minutes to solubilise the inner membranes. The suspension was centrifuged at 10000g for 10 minutes to remove aggregates, the supernatant was further centrifuged at 50000g for 3 hours. The pellet, containing the outer membranes

was washed in PBS and resuspended in the same buffer. Protein concentration was measured by the D.C. Bio-Rad Protein assay (Modified Lowry method), using BSA as a standard.

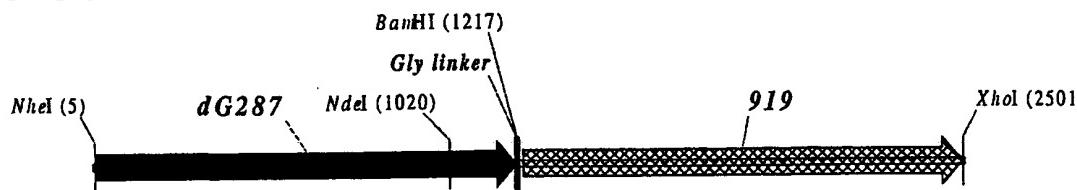
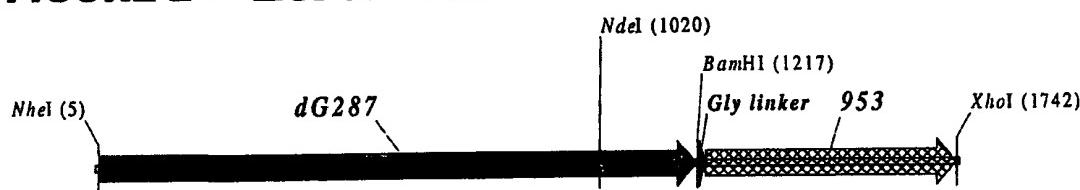
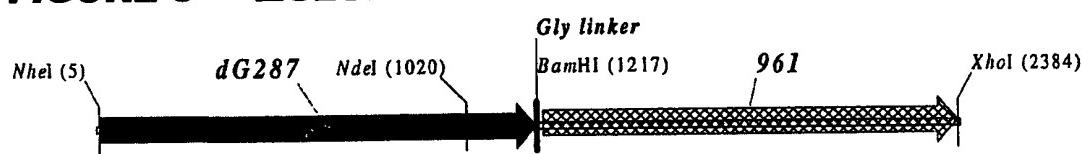
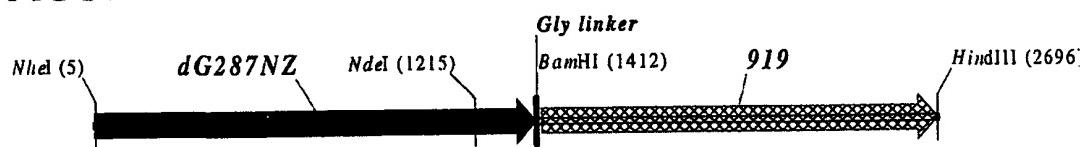
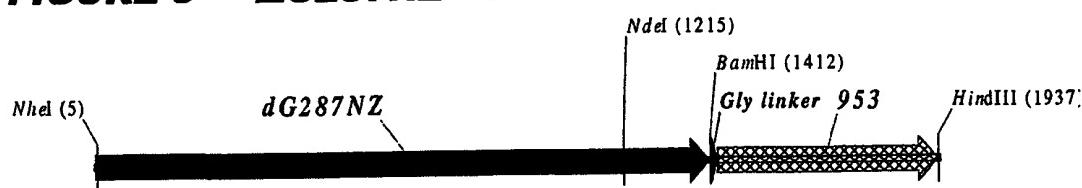
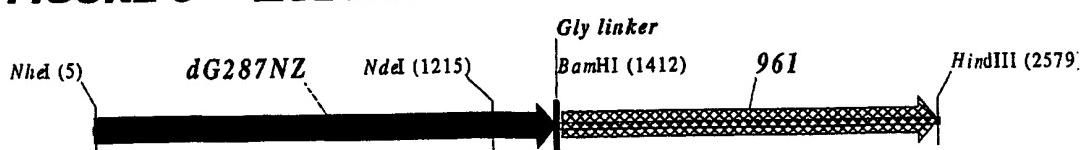
Total cell extracts were prepared as follows: *N. meningitidis* strain 2996 was grown overnight on a GC plate, harvested with a loop and resuspended in 1ml of 20mM Tris-HCl.

- 5 Heat inactivation was performed at 56°C for 30 minutes.

CLAIMS

1. A method for the simultaneous heterologous expression of two or more proteins of the invention, in which (a) two or more proteins of the invention are fused.
2. The method of claim 23, in which the two or more proteins are: (a) 919 and 287; (b) 953 and 287; (c) 287 and ORF46.1; (d) ORF1 and ORF46.1; (e) 919 and ORF46.1; (f) ORF46.1, 287 and 919; (g) 919 and 519; and (h) ORF97 and 225.
5
3. The method of claim 24, in which 287 is at the C-terminal end of protein (a), (b) or (c).
4. The method of any preceding claim, in which the expression is in an *E.coli* host.
5. A protein expressed by the method of any preceding claim.
- 10 6. A hybrid protein of formula NH₂-A—B-COOH, wherein A and B are different Neisserial proteins.
7. The protein of claim 6, wherein A and B are each selected from orf1, orf4, orf25, orf40, orf46, orf83, 233, 287, 2921, 564, 687, 741, 907, 919, 953, 961 and 983.
8. The protein of claim 7, wherein A and B are each selected from ORF46.1, 287, 741, 919,
15 953, 961 and 983.
9. The protein of claim 8, wherein at least one of said ORF46.1, 287, 741, 919, 953, 961 and 983 is used in essentially full-length form
10. The protein of claim 8 or claim 9, wherein at least one of said ORF46.1, 287, 741, 919, 953, 961 and 983 has a deletion.
- 20 11. The protein of claim 10, wherein A and/or B has a poly-glycine deletion ('ΔG').
12. The protein of claim 11, wherein A and/or B is ΔG-287, ΔGTbp2, ΔG741, or ΔG983.
13. The protein of claim 10, wherein A and/or B is a truncated protein.
14. The protein of claim 13, wherein A and/or B is Δ1-287, Δ2-287, Δ3-287 or Δ4-287.
15. The protein of claim 10, wherein a domain of A and/or B is deleted.

16. The protein of claim 15, wherein A and/or B is 287B, 287C, 287BC, ORF461-433, ORF46433-608, ORF46, or 961c.
17. The protein of claim 6, wherein A and B are: (a) 919 and 287; (b) 953 and 287; (c) 287 and ORF46.1; (d) ORF1 and ORF46.1; (e) 919 and ORF46.1; (f) ORF46.1 and 919; (g) 919 and 519; or (h) ORF97 and 225.
5
18. The protein of claim 17, wherein the protein is ΔG287-919, ΔG287-953, ΔG287-961, ΔG983-ORF46.1, ΔG983-741, ΔG983-961, ΔG983-961C, ΔG741-961, ΔG741-961C, ΔG741-983, ΔG741-ORF46.1, ORF46.1-741, ORF46.1-961, ORF46.1-961C, 961-ORF46.1, 961-741, 961-983, 961C-ORF46.1, 961C-741, 961C-983, 961CL-ORF46.1, 10 961CL-741, or 961CL-983.
19. The protein of claim 8, wherein A or B is 287.
20. The protein of claim 19, wherein B is 287
21. The protein of claim 19, wherein A is ΔG-287
22. The protein of claim 21, wherein B is ORF46, 919, 953 or 961.
- 15 23. The protein of any one of claims 19 to 22, wherein 287 is from strain 2996 or 394/98.
24. The protein of claim 8, wherein A is 961.
25. The protein of any one of claims 6 to 24, wherein A and B are from the same strain.
26. The protein of any one of claims 6 to 24, wherein A and B are joined directly
27. The protein of any one of claims 6 to 24, wherein A and B are joined via a linker peptide.
- 20 28. The protein of claim 27, wherein the linker peptide is a poly-glycine linker, with the proviso that B is not a ΔG protein.

FIGURE 1 — Δ G287—919**FIGURE 2 — Δ G287—953****FIGURE 3 — Δ G287—961****FIGURE 4 — Δ G287NZ—919****FIGURE 5 — Δ G287NZ—953****FIGURE 6 — Δ G287NZ—961**

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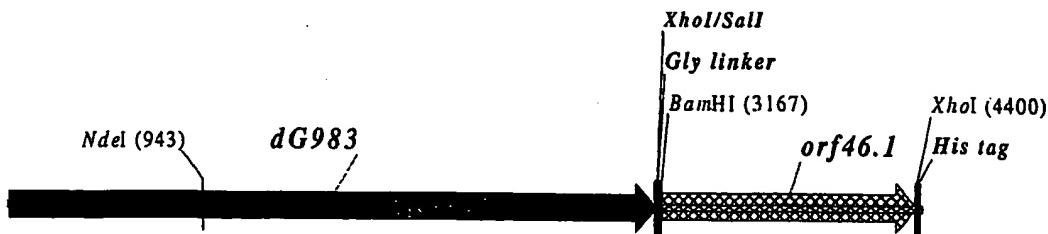
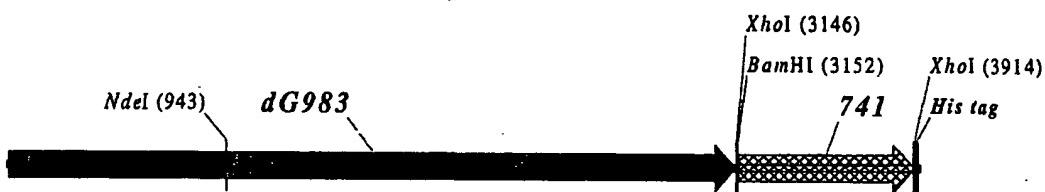
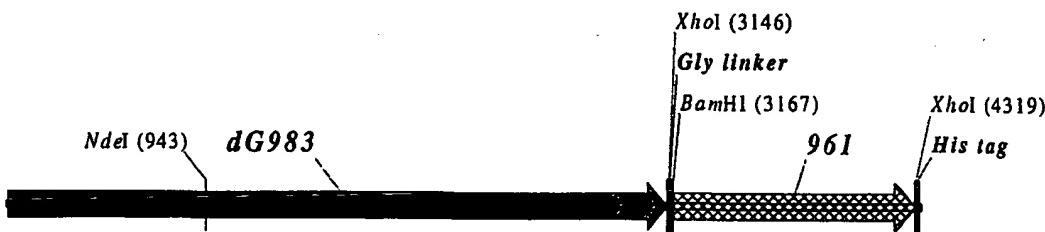
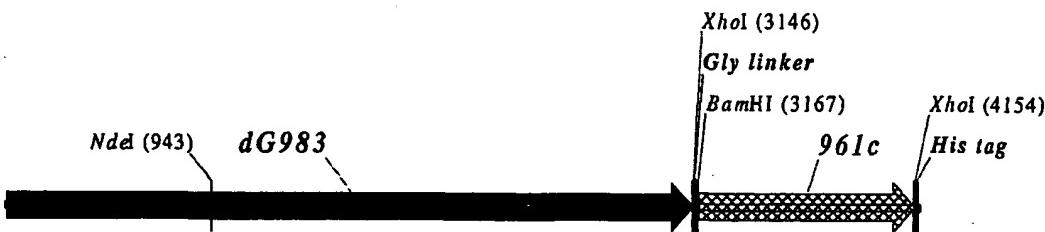
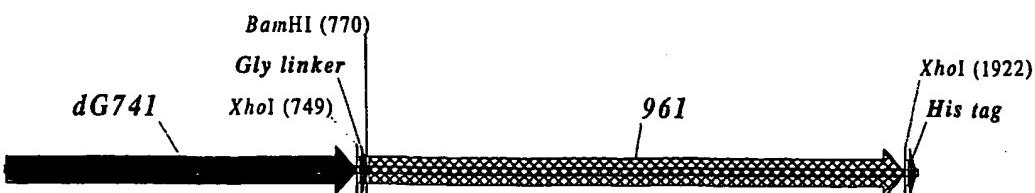
FIGURE 7 — $\Delta G983$ -ORF46.1**FIGURE 8 — $\Delta G983$ -741****FIGURE 9 — $\Delta G983$ -961****FIGURE 10 — $\Delta G983$ -961c****FIGURE 11 — $\Delta G741$ -961**

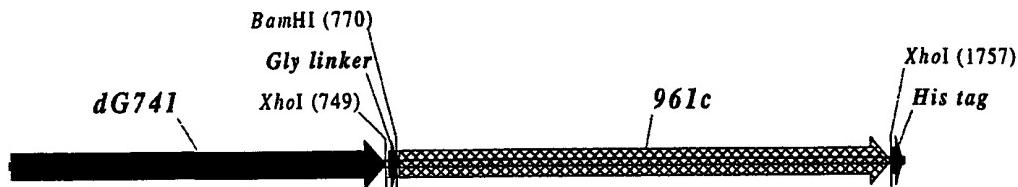
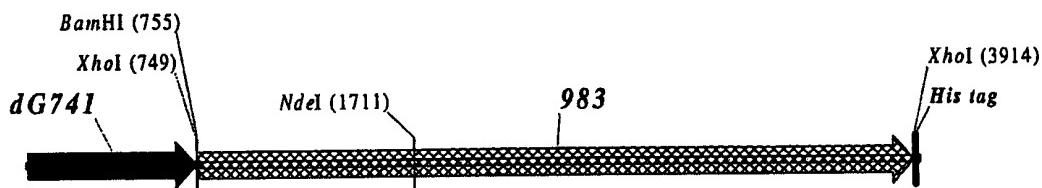
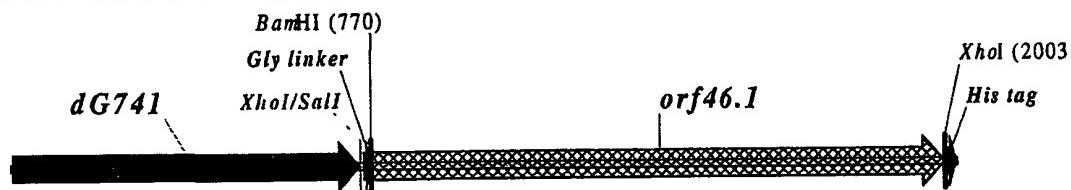
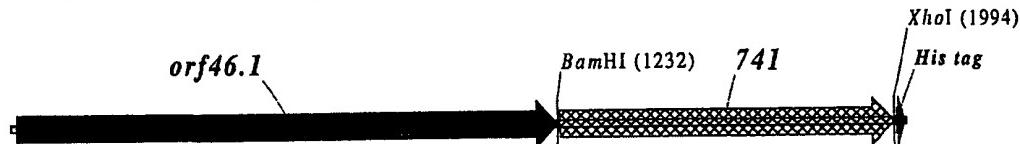
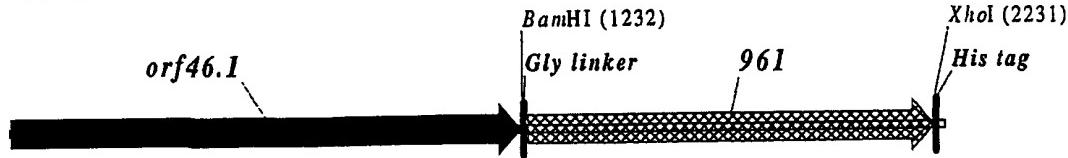
FIGURE 12 — $\Delta G741\text{-}961c$ **FIGURE 13 — $\Delta G741\text{-}983$** **FIGURE 14 — $\Delta G741\text{-}ORF46.1$** **FIGURE 15 — ORF46.1-741****FIGURE 16 — ORF46.1-961**

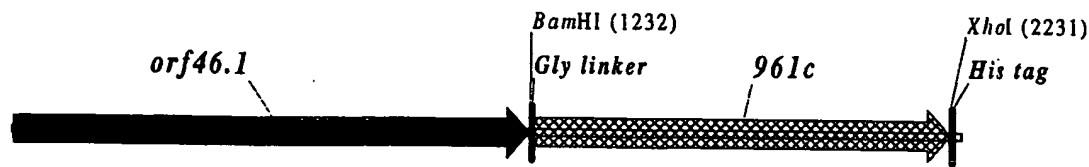
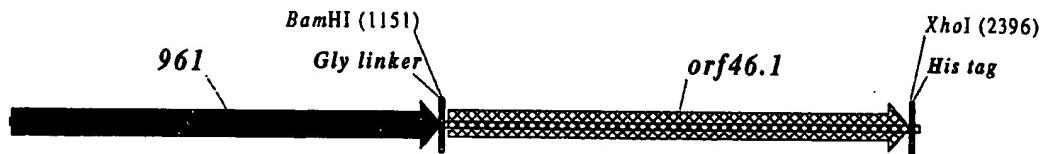
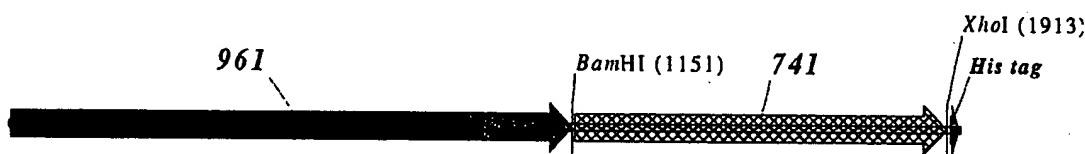
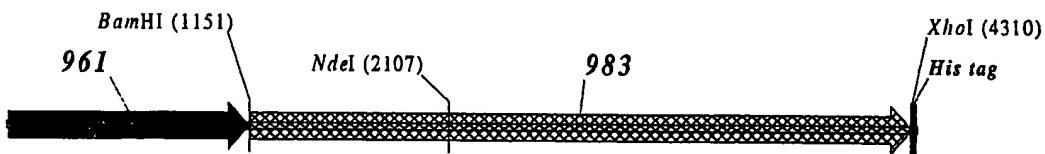
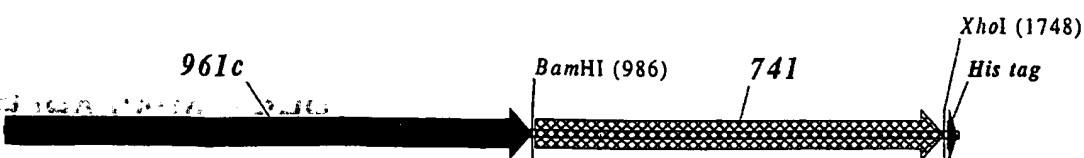
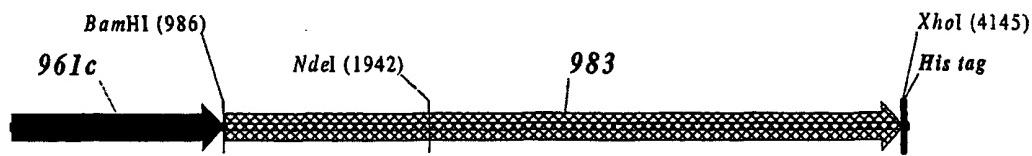
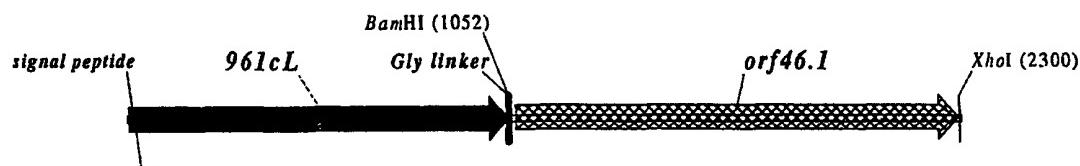
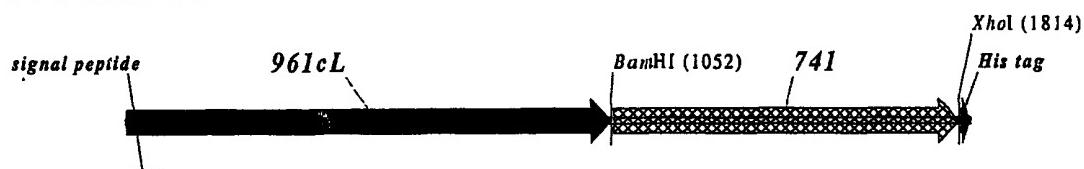
FIGURE 17 — ORF46.1—961c**FIGURE 18 — 961-ORF46.1****FIGURE 19 — 961-741****FIGURE 20 — 961-983****FIGURE 21 — 961c-ORF46.1****FIGURE 22 — 961c-741**

FIGURE 23 — 961c-983**FIGURE 24 — 961cL-ORF46.1****FIGURE 25 — 961cL-741****FIGURE 26 — 961cL-983**